Seaweed cultivation in the Faroe Islands

An investigation of the biochemical composition of selected macroalgal species, optimised seeding technics, and open-ocean cultivation methods from a commercial perspective

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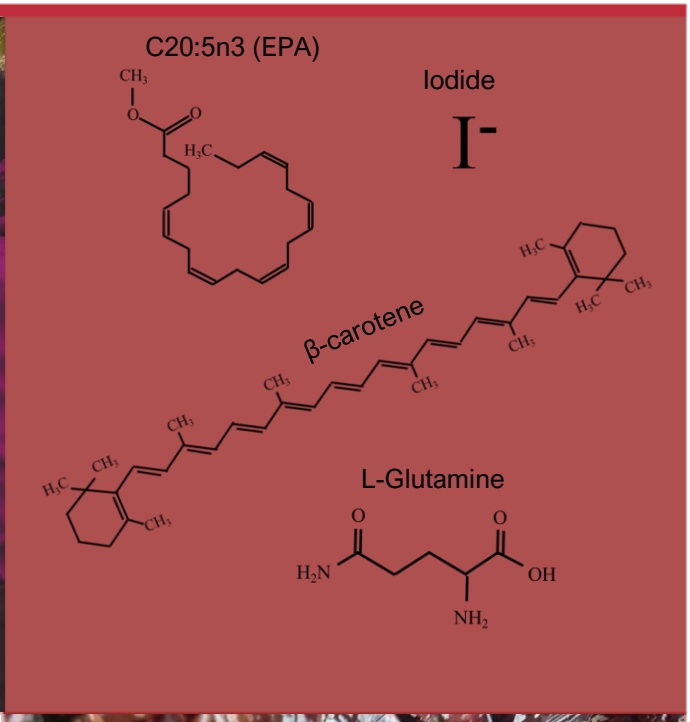

Urd Grandorf Bak

Industrial PhD Thesis

February 2019

DTU Food
National Food Institute

OCEAN rainforest

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PhD thesis by Urd Grandorf Bak

National Food Institute

Technical University of Denmark

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DATA SHEET

Title: Seaweed cultivation in the Faroe Islands - An investigation of the biochemical composition of selected macroalgal species, optimised seeding technics, and open-ocean cultivation methods from a commercial perspective

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Summary

A global need for sustainable produced biomass has kick-started the development of commercial macroalgal cultivation in Europe. In contrast to the well-established nearshore cultivation in Asia, a Faroese company, Ocean Rainforest, has developed a MacroAlgal Cultivation Rig (MACR) that is suitable for oceanic-conditions (>50 m water depth). The design has proven itself scalable since 10 MACR's have been in operation since 2010. Still, the challenge is to reduce the cost of production to be competitive within the global market.

The purpose of this PhD project was to optimize the cultivation method at sea for an applicable and economically profitable industry. The kelps *Saccharina latissima* and *Alaria esculenta* were cultivated on a commercial scale with 40 km of growth lines deployed in the Faroe Islands. The aquaculture output (AO; yield/ha/year) was optimised and multiple partial harvesting method was tested. Growth and yield were monitored through a three-year period at two cultivation sites, and the biochemical composition was determined from *S. latissima, A. esculenta* and *Laminaria digitata* samples collected monthly (2015-2016). Also, the purpose was to obtain in-depth knowledge about the target biochemical composition of cultivated brown macroalgal species to plan harvest, decide on product applications, and plan for future biorefinery processes. Analyses of dry matter, minerals (ash), protein, carbohydrate, lipid, amino acid profile, fatty acid profile, antioxidant activity, α-carotene, β-carotene and D-vitamin, iodine, lead, mercury, cadmium and arsenic including inorganic arsenic were performed, and evaluated in relation to their interannual, seasonal, site and depth variations. Finally, the purpose was to obtain new optimised seeding techniques of two kelp species and to find a way to the more challenging seeding and obtaining biomass of the red macroalga *Palmaria palmata*.

Saccharina latissima sporophytes were seeded directly on ribbons using a binder and the method showed same length and density as when using the more traditional "twine around rope" method. Also, the cost was reduced by 13% or 23% by using this direct seeding method on ribbons or nylon ropes, respectively. *Palmaria palmata* was successfully direct seeded for the first time, though having a low density. A very high density was obtained by using natural seeding over winter from nets placed on wild populations.

The results of the biochemical composition showed a significant variation of several compounds between species and seasons, but not between years, cultivation depths and sites. There was a seasonal trend that dry matter and carbohydrates concentrations went up in winter where ash and protein were lowest, and the opposite during summer. *Saccharina latissima* had high carbohydrate (42-57% of dw) and ash concentration (36-42% of dw), lower content of protein (11-14% of dw), and small amounts of lipids (2.4% of dw). Vitamin D and α-carotene were not detected in any of the species and a low antioxidant activity was revealed. β-Carotene was found at 10-22 ppm of dw. Iodine concentration was high for *S. latissima* and *L. digitata* (3998-5361 ppm of dw) and lower for *A. esculenta* (234 ppm of dw) with lowest concentration during harvest season. The levels of heavy metals found within this work were in similar range as other food sources and below threshold values. The results can be used directly as product documentation and the results revealed that depth and site variation were not significant for the biochemical composition. The results made a great contribution to the better understanding of the biochemical composition of the cultivated macroalgae and that important variables are species and season.

For the first time, multiple partial harvesting was proven successful for *S. latissima* and *A. esculenta* with up to five harvests of the same biomass without reseeding. *Saccharina latissima* was suitable for two harvests per year and *A. esculenta* could be harvested once a year. In the second-year of cultivation fouling of other macroalgal species occurred on the lines and self-seeded *L. digitata* could also be harvested. The multiple partial harvesting alone reduced the operational cost of the MACR from € 36.73 to less than € 9.27 per kg dry weight (dw) of cultivated *S. latissima*. The 10 m vertical growth lines seeded with *S. latissima* had a mean yield of 0.29 kg dw per meter per harvest when four partial harvests were made over a two-year period. The AO was found to be 1.4-4 tonnes dw per hectare per year (including handling space). The mean yield and AO are one of the first of its kind under open-ocean cultivation with commercial-scale testing and without extrapolating data from research trials. This work has thus optimised commercial kelp cultivation methods in the Faroe Islands.

Resumé

Et globalt stigende behov for bæredygtigt produceret biomasse har igangsat kommercielle aktiviteter for at etablerer en europæiske tang-dyrknings-industri. I modsætning til den veletablerede kystnære dyrkning i de asiatiske lande, har den færøske virksomhed Ocean Rainforest udviklet et dyrkningssystem (MacroAlgal Cultivation Rig; MACR), der er egnet til dyrkning under oceaniske forhold (> 50 m vanddybde). Dyrkningssystemet kan nemt opskaleres og 10 MACR har været i drift siden 2010. Udfordringen er dog at reducere produktionsomkostningerne, så tangdyrkning er konkurrencedygtigt globalt.

Formålet med dette ph.d.-projekt var derfor at optimere dyrkningsmetoderne på havet og sænke omkostningerne ved produktion. De to store brunalgearter *Saccharina latissima* og *Alaria esculenta* blev dyrket kommercielt med mere end 30 km vækstliner udsat på Færøerne. Produktionsudbyttet (kg / ha / år) blev optimeret ved i felten at teste flere høst fra samme line per år i flere år i træk. Vækst og udbytte blev målt over en treårig periode på to dyrkningssteder, og tangens indholdsstoffer blev bestemt fra månedligt indsamlede tangprøver (2015-2016). Desuden var formålet at opnå indgående kendskab til de biokemiske indholdsstoffer af de dyrkede tangarter for bedre at kunne planlægge høst, bestemme egnede produkt muligheder og for bedre at kunne planlægge fremtidige bioraffinaderi-processer. Tangprøverne blev analyseret for mineraler (aske), proteiner, kulhydrater, lipider, tørstof, aminosyreprofil, fedtsyreprofil, antioxidanter, vitaminindhold (a-caroten, β-caroten og D-vitamin), jod og tungmetaller (bly, kviksølv, cadmium, arsen og uorganisk arsen). Alle disse indholdsstoffer blev evalueret i forhold til deres årstidsvariation, mellem forskellige år, dyrkningssteder og dyrkningsdybder. Desuden var formålet at opnå nye og bedre såningsmetoder for de store brune makroalger og at udvikle en metode som muliggør såning af den mere udfordrende røde tangart *Palmaria palmata*.

Dette arbejde bevidste at flere høstninger med genvækst fra samme line er muligt for arterne *S. latissima* og *A. esculenta,* og fem gentagende beskæringer tangen var muligt for *S. latissima* med to beskæringer om året i tre år i træk, og for *A. esculenta* én gang om året to-tre år i træk. Flere høst per såning sænkede installations- og såningsomkostningerne per udsat MACR fra 275 kr. per kg dyrket *S. latissima* til mindre end 75 kr. per kg. I det andet vækstår forekom der

begroning af arten *Laminaria digitata* og da den også er en kommerciel interessant tangart, blev den også høstet og indholdsstofferne analyseret. *Saccharina latissima* havde et gennemsnitligt udbytte på 0,29 kg (tørvægt) pr. meter pr. høst, baseret på et gennemsnit af udbyttet fra 5 km tangliner dyrket fra overfladen og 10 meter ned, hvor linerne blev høstet fire gange over en toårig periode. Produktionsudbyttet var 1,4-4 tons tørvægt pr. hektar pr. år (inklusiv arealet anvendt til at håndtere linerne). Resultatet er det første af sin slags, da kommerciel tangdyrkning på åbent hav på stor vanddybde ikke er muliggjort før.

Tangens biokemiske indhold varierede mellem arter og årstider, men generelt ikke imellem år, dyrkningsdybde eller dyrkningssteder. Der var en sammenhæng mellem tørstof- og kulhydratkoncentrationerne, som steg i vinterhalvåret, hvor aske og protein viste sig at være lavest. Omvendt var aske og protein højest i sommerhalvåret, hvor tørstof og kulhydrater var lavest. *Saccharina latissima* havde et højt indhold af kulhydrater (42-57% tørvægt) og aske (36-42% tørvægt), et noget lavere proteinindhold (11-14% tørvægt) og små mængder lipider (2,4% tørvægt). Antioxidant-indholdet var lavt, og D-vitamin og a-caroten var ikke målbart. β-Caroten var tilstede med omkring 10-22 ppm tørvægt. Jodkoncentrationen var høj for *S. latissima* og *L. digitata* (3.998-5.361 ppm dw) og markant lavere for *A. esculenta* (234 ppm dw). Tungmetalindholdet var på niveau med andre fødevarekilder og under tærskelværdier.

Saccharina latissima sporophytter blev sået direkte på flade båndlignede reb ved hjælp af et bindemiddel. Væksten var den samme, som for tang der var sået på såliner og dyrket i tanke indtil en synlig størrelse, og derefter snoet om reb inden udsætning. Såningsomkostningerne blev reduceret med 13% eller 23% ved at anvende direkte såning på henholdsvis bånd eller nylonreb. *Palmaria palmata* tetrasporer blev for første gang sået ved at anvende den direkte såningsmetode, men med lav tilvækst og densitet. Et forsøg med naturlig såning af et dyrkningsnet over vinteren viste tæt såning med 5 kg pr. m^2 *P. palmata* i maj måned.

Dette arbejde har således optimeret kommerciel tangdyrkning på Færøerne og bevist potentialet for flerårige høst af tangen. Den dybdegående undersøgelse af tangens indholdsstoffer er direkte anvendeligt til produktdokumentation til kunder. Endeligt har resultaterne fra dette projekt bragt tangdyrkning et skridt videre mod en ny industri og indtægtskilde for Færøerne.

Preface

This PhD was funded by the Innovation Fund Denmark on the day I gave birth to my second child, June 30, 2015. Consequently, it was initiated with a six months delay, January 2016. This Industrial PhD was made in collaboration between the National Food Institute, Technical University of Denmark (DTU Food), and the company Ocean Rainforest Sp/F. My background was a master's degree in Environmental Biology and Geography from Roskilde University, Denmark.

Before the funding grant, I was employed by the Faroese company Ocean Rainforest and DTU Food as research assistance both places. From November 2014 until May 2015, I developed the growth monitoring and biomass sampling program at Ocean Rainforest, and first monitoring was therefore initiated already March 2015, before this PhD initiated. During my maternity leave, the staff at Ocean Rainforest continued the monitoring and sampling program according to my instructions.

This Industrial PhD work includes many biochemical analyses, which I did not perform, except pre-treatment, some dry matter determination, freeze-drying and grinding work. Specialist laboratory staff and students at DTU Food made the analyses of amino acids, nitrogen, vitamins, inorganic arsenic, and iodine concentration of the macroalgal samples. Lipid, fatty acids, protein, polyphenols, antioxidants, metals and carbohydrates were analysed by the research institute Mátis (Iceland), as part of the research and innovation project called MacroValue funded by the Nordic Innovation, 2015-2018. Total phosphorus and carbon were analysed by Aarhus University as part of the project MAB4, which was funded by the Innovation Fund Denmark.

This work also includes seeding of kelps. This work was made in close collaboration with the seaweed breeding company Hortimare, the Netherlands, and the knowledge sharing was restricted by NDA. A small part of the seeding material (for seeding of 2 km growth lines) was produced in Kaldbak, the Faroe Islands, by me and the remaining seeding material was made by Hortimare (always using reproductive material of Faroese seaweed). The seedings onto ropes and deployments of these were always done by Ocean Rainforest. Because of the NDA of Hortimare, I will not describe results found in the work regarding spore release and the

maintenance and upscaling of the microscopic gametophyte life stage of the seaweed. Instead, other aspects of the seeding optimisation will be included.

The operation at Ocean Rainforest spans an unbroken chain from seeding, cultivation, harvesting and processing into storage stable intermediates. The PhD include only the biomass production part (**Figure ix)** and does not treat the processing and marketing part of the production chain.

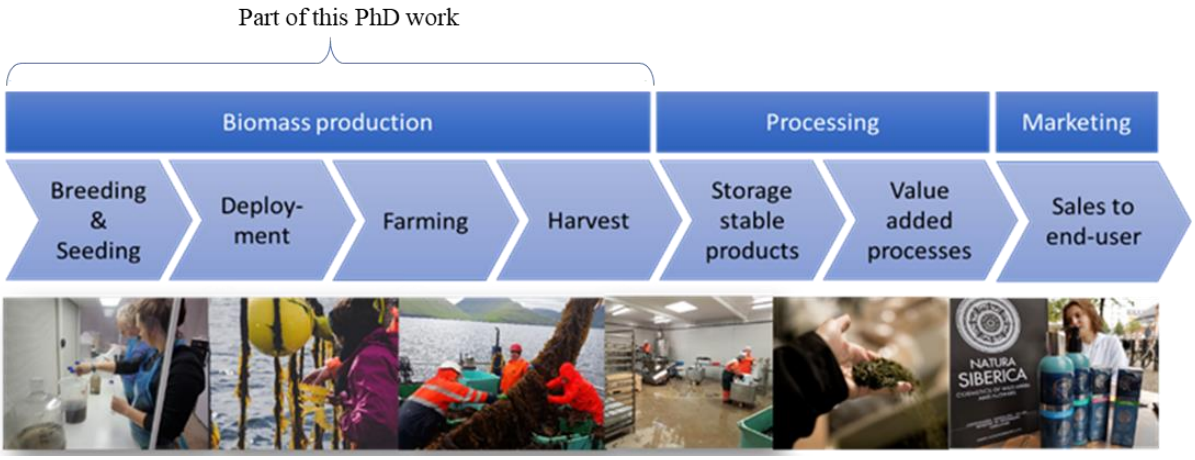


Figure ix The seven operation phases that are included in the operational activities of Ocean Rainforest, and the overall processes are “biomass production”, “processing” and “marketing”. This PhD will target the biomass production and not the other processes.

Background of the Company

The company Ocean Rainforest operates a seaweed production in the Faroe Islands, and cultivation takes place in the fjord Funningsfjørður in the North of Eysturoy. The company has developed an innovative system for macroalgal cultivation in open-oceans. When this project was initiated in 2016, the cultivation capacity at sea was 10 km of seeded lines on five cultivation systems; today, three years later, it is 30 km of seeded lines on eight cultivation systems. The annual production capacity is ~180 tonnes fresh weight and the harvested biomass is being sold to the business-to-business market.

An important strategic activity for the company has been to participate in relevant research and development projects on a national, regional and international level. The objective with these projects and also the involvement of this Industrial PhD work has been to further develop the industry towards economically feasible cultivation techniques and economically viable concepts of innovative food additives, cosmetics, pharmaceutical, marine proteins feed and biofuels. Ocean Rainforest currently operates with a permit on the Bakkafrost fish farming license area A71 and A02 in Funningsfjørður, Faroe Islands. On an international scale, the top management of Ocean Rainforest is investigating how to acquire licenses for macroalgal cultivation in Europe and North America.

The company has three full-time employees, and a call-on crew to help with seeding, harvesting and processing. The company has a Board of Directors and is owned by locals, who are also involved in the activities of the company. The Managing Director is Ólavur Gregersen who is co-founder and major shareholder, but also the industrial supervisor of this PhD work.

All the work at sea is made from the boat M/B Tongul FD 87 (**Figure xi**) that is constructed with steel and is 12.2 m long and 5 m deep. M/B Tongul has a crane that can lift 2-3 tonnes and a winch of similar tow capacity. The company has a 620 m^2 processing plant in Kaldbak, Faroe Islands, and the building has a capacity for cleaning, packaging, freezing and drying as well as office facilities and laboratories (**Figure xi**). Furthermore, a hatchery has been installed for "coil nursery" and other seeding activities. The Ocean Rainforest processing plant in Kaldbak is certified by The Faroese Food Authority for food production.


Figure xi The boat Tongul working with deployment of cultivation lines in a nearshore exposed site in Funningsfjørður **(left)** and the newly installed laboratory in Kaldbak used for sample preparation and production of seeding material **(right)**.

The weather conditions in the Faroe Islands can make it impossible to operate at sea for weeks, and often demands a plan B during operation. Also, the work in the laboratory and in the processing plant with either seeding material or freshly harvested material required strong adaptability due to the early stage of operation.

Acknowledgement

This Industrial PhD the first Industrial PhD in a Faroese company funded through grants from the Innovation fund Denmark. I am very grateful for this opportunity and financial funding.

Initially, I would like to thank my university supervisors Susan L. Holdt and Charlotte Jacobsen for always being helpful and patient with me. Thank you for teaching me, an environmental biologist, about food chemistry, analytical methods and the nutritional perspectives of algae. During my master thesis, Susan taught me how to cultivate – and eat – seaweed and she helped me stay in the algae field until this PhD was successfully funded. I am grateful for that!

Secondly, I would like to thank my colleagues in the research group Bioactives – Analysis and Application for welcoming me at DTU Food. A special thanks go to the people who have helped with the analyses of nutritional components: Anette Landin, Birgitte Herbst, Cecilie Wirenfeldt, Goncalo S. Marinho, Jens J. Sloth, Jette Jakobsen, Julie Høgsbro, Katharina Johanna Kreissig, Karoline Havkrog and Kenneth Skaaning.

Concerning Ocean Rainforest, my warmest gratitude goes to all of you in and around the company: Andrias Hammer, Askur Gregersen, Elsa Berg, Heri Arge, Jacob Højgaard, Ludwig Schmidtchen, Magni Arge, Manbjørn í Grund, Nicoline Korsvold, Petur Arni Kristiansen, Steinbjørn í Dali, Silja Gregersen, Unn Laksá and Olavur Ellefsen. Thank you for welcoming me and my family to your beautiful country and bringing us close to the culture of the Faroe Islands. Thank you for teaching me the skills of a seaman, and for being loyal to all my crazy ideas. A special thanks to Katrin Gregersen who I admire for her persistent helpfulness and optimism. Most of all, I would like to thank Ólavur Gregersen, my industrial supervisor, for a close, fun and educational collaboration.

Though the work at Ocean Rainforest, I met many great people and would like to thank project partners for fruitful collaboration, and especially Job Schipper and colleagues from Hortimare, Rósa Jónsdóttir and colleagues from Matis, Annette Bruhn and colleagues from Aarhus University - Bioscience, and Javier Infante from Patagonia Seaweeds.

A special thanks go to my mother Eva Steendahl and mother-in-law Mette Bak who countless times have taken care of our daughters. I want to thank my aunt-in-law Elsebeth Melby for

helpful proofreading. The final and warmest gratitude to those of you closest to me – my husband Jens Bak, and our daughters Yrsa and Viol.

I dedicate this work to Viol as she was raised along with my PhD education.

Urd Grandorf Bak, February 2019

List of Publications

The papers are appended to the thesis, and in the main text, they will be referred to by their Roman numerals. For simplicity, they will all be referred to as papers even that some of them still appear as submitted manuscripts or manuscripts in preparation. All papers are reproduced with permission of the publisher and with permission of the co-authors.

Publications

PAPER I	**Urd Grandorf Bak**, Agnes Mols-Mortensen and Ólavur Gregersen (2018). Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting. *Algal Research* 33 (2018) 36-47. https://doi.org/10.1016/j.algal.2018.05.001
PAPER II	**Urd Grandorf Bak**, Javier Infante and Ólavur Gregersen (2019). Offshore seaweed cultivation in the past, present and in future. *Botanica Marina*, book chapter for the special issue: *Seaweed Resources of the World - A 2020 Vision*. Submitted (Jan 2019).
PAPER III	**Urd Grandorf Bak**, Cecilie Wirenfeldt Nielsen, Gonçalo Silva Marinho, Ólavur Gregersen, Rósa Jónsdóttir and Susan Løvstad Holdt (2018). The seasonal variation in nitrogen, amino acid, protein and N-to-Protein conversion factors of commercially cultivated Faroese *Saccharina latissima* and evaluation of the use for food and feed. *Algal Research.* Submitted (Oct 2018), resubmitted (Feb 2019).
PAPER IV	**Urd Grandorf Bak**, Kenneth Skaaning, Rie Romme Rasmussen, Susan Løvstad Holdt, Ólavur Gregersen and Jens J. Sloth (2019). Seasonal, site and depth variation and chemical risk evaluation of total iodine in offshore commercially cultivated Faroese *Saccharina latissima*, *Alaria esculenta* and *Laminaria digitata*. Draft intended for *Journal of Applied Phycology*.

List of Abbreviations

AO	aquaculture output
CEN	European Committee for Standardization
dw	dry weight
EPA	eiosapentaenoic acid
FAME	fatty acid methyl esters
FAO	Food and Agriculture Organization of the United Nations
MACR	macroalgal cultivation rig
MBSL	meters below sea level
NPP	net primary production
ORAC	oxygen radical absorbance capacity
PERMANOVA	permutational ANOVA
RGR	relative growth rate
SD	standard deviation
SDG	UN Sustainable Development Goals
SGR	specific growth rate
ww	wet weight

Authorship of species

Alaria esculenta	(Linnaeus) Greville
Ascophyllum nodosum	(Linnaeus) Le Jolis
Cladophora rupetris	(Linnaeus) Kützing
Cystoseira crinita	Duby
Fucus serratus	Linnaeus
Fucus vesiculosus	Linnaeus
Laminaria digitata	(Hudson) J.V. Lamouroux
Laminaria hyperborea	(Gunnerus) Foslie
Macrocystis perifera	(Linnaeus) C. Agardh
Palmaria palmata (Rhodymenia palmata)	(Linnaeus) O. Kuntze
Pelvetia canaliculata	(Linnaeus) Decaisne and Thuret
Saccharina japonica (Laminaria japonica)	(Areschoug) C.E. Lane, C. Mayes, Druehl and G.W. Saunders
Saccharina latissima (Laminaria saccharina)	(Linnaeus) C.E. Lane, C. Mayes, Druehl and G.W. Saunders
Undaria pinnatifida	(Harvey) Suringar

Table of Contents

1 Introduction

Macroalgae (seaweeds) have the potential to be able to solve many of today's most pressing environmental and social sustainability challenges. These challenges are ranging from a growing demand for food and feed, a need for health and pro- and prebiotic promoting products for the human wellbeing and non-fuel-based carbohydrates to an insatiable market for biofuel, bioplastics and textiles. Furthermore, a globally increased macroalgal production could be a significant step towards achieving the UN Sustainable Development Goals (SDG): on sustainable consumption and production (SDG 12), on climate change (SDG 13), and on sustainable exploitation of life below water (SDG 14), as macroalgae are suggested as a tool against climate change, improve ocean health and preserve ocean life (**Figure 1.1**).

Figure 1.1 The UN Sustainable Development Goals and how macroalgal cultivation could meet these goals.

Macroalgae sit at the bottom of the underwater food chain and only need sunlight, carbon dioxide and nutrients (such as nitrogen and phosphorus) to grow (Hurd et al. 2014, Levine 2016). All these necessities are most often in abundant free-supply in the ocean. Unlike other seafood or livestock farming, macroalgae do therefore not rely on feed or fertiliser for growth.

Natural macroalgal beds have an important function for marine ecosystems, and cultivated macroalgal plots can rapidly promote biodiversity including many fish species (North 1987, Skjermo et al. 2014, Hasselström et al. 2018). Cultivated macroalgae can thus provide nursery areas to various species, and appropriately designed cultivation structures can, therefore, serve as additional communities and provide ecosystem services that need to be valued appropriately

(Chopin 2014, Buck et al. 2017, Buschmann et al. 2017). The value of the ecosystem service that is provided by natural macroalgal beds has been estimated to US$ 1.1-2.9 million per km^2 per year (Costanza et al. 2014, Buschmann et al. 2017). As these numbers were estimated for natural macroalgal beds, valid extrapolations of large-scale cultivation are needed to determine their true impact.

Besides adding new habitats, large-scale macroalgal cultivation has the potential to considerably mitigate climate change and ocean acidification (**Figure 1.2**). Currently, the Earth's net primary production (NPP) mainly origins from microalgae growth in the open-ocean and from plants in the tropical rainforest. However, the average NPP of macroalgae, measured as gram per square meter per year, is higher than NPP of the plants in the tropical rainforest (**Figure 1.2**).

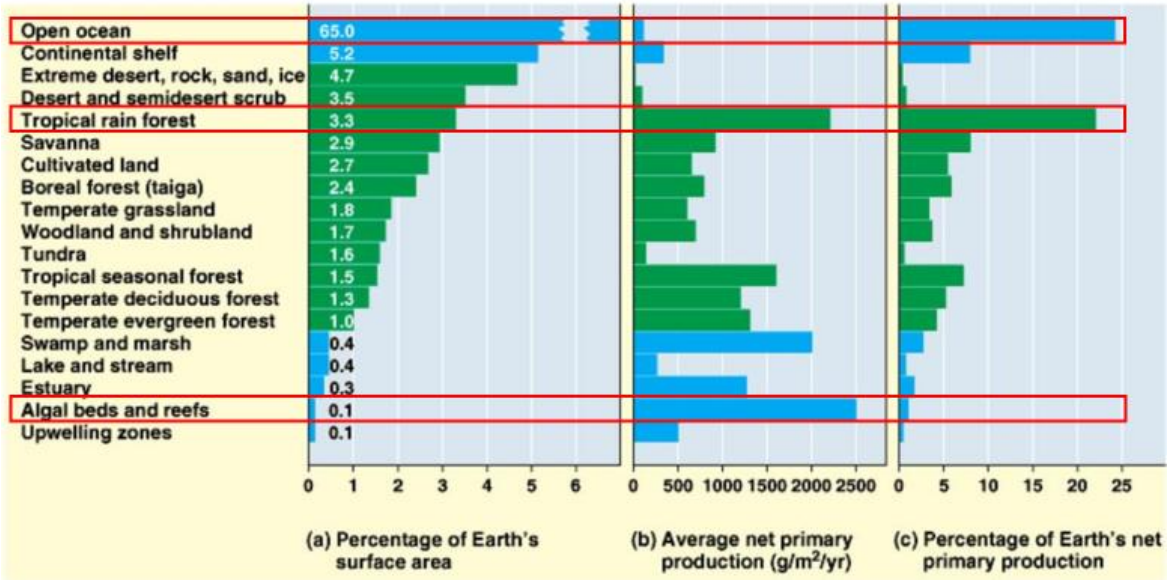


Figure 1.2 Comparison of the Earth's Net Primary Production (NPP) with "open-ocean", "tropical rainforest" and "algal beds and reefs" highlighted for comparison of **(a)** percentage of Earth's surface area, **(b)** average net primary production (g/m^2/year), and **(c)** percentages of Earth's net primary production (© Person Education Inc. publishing as Benjamin Cummings, 2002).

In order to increase the Earth's NPP, the need is to expand the highly efficient carbon fixation from macroalgal beds to the large area of open-ocean. This can be done when a substrate is seeded and provided in the open-ocean.

Macroalgal biomass is rich in bioactive compounds as vitamins, pigments and antioxidants (Holdt and Kraan 2011), giving it a superfood status and making it an important natural ingredient in cosmetics and pharmaceutical products. Also, macroalgae have a high fibre and

mineral content – and for some species, high-iodine content – together with essential amino acids and omega-3-fatty acids make the biomass a suitable food and feed source. The current use of macroalgae is mainly for human consumption products (82%) followed by pharmaceuticals and cosmetics (12.2%), animal feed (2.9%), and lastly agriculture (2.6%) as fertilizer products (Ferdouse et al. 2017).

Today, 96% of the global macroalgal production is cultivated and only 4% is wild harvested. A total of 33 countries are registered by the Food and Agriculture Organization of the United Nations (FAO) to have a macroalgal production, where China, Japan, Indonesia, the Philippians and Korea are world leaders in terms of quantities produced and account in total for more than 95% (Ferdouse et al. 2017).

In 2015, cultivated macroalgal accounted for 29.4 million tonnes wet weight (ww) globally, worth €4.92 billion, and the macroalgal biomass represented the largest group of mariculture organisms (FAO 2016, Brunswick 2017, Ferdouse et al. 2017). Macroalgal cultivation is the fastest growing form of aquaculture, and the production is expected to double in the next ten years (by 2025; Cottier-Cook 2016, Buschmann et al. 2017, Ferdouse et al. 2017).

The western world (Europe and North America) has a long tradition of excellent research in phycology, but little experience in commercial macroalgal cultivation. Currently, the cost of commercial cultivation in the western world exceeds the sale price and a profitable operation is a challenge (Fernand et al. 2017). To solve this bottleneck innovative methods and research are needed (Charrier et al. 2017, Fernand et al. 2017). The cultivation in China has an aquaculture output (AO) of 97.4 tonnes ww per hectare per year when including all species cultivated (Roesijadi et al. 2008). This AO is a state-of-the-art productivity that should be met, or surpassed, in future farming initiatives in the western world.

The North Atlantic macroalgal cultivator, Ocean Rainforest, has developed a MacroAlgal Cultivation Rig (MACR) that is suitable for open-ocean environments. Since 2010, the rig has been tested and scaled up, and has proven to be resilient to unpredictable and harsh ocean conditions. Today, Ocean Rainforest operates a value chain that spans from seeding, cultivation, harvesting and pre-processing into storage stable products for the food, feed and cosmetic markets. The company is today one of the leading operations of its kind in Europe.

To get this far, ground-breaking research and operational optimisation were needed within the below-mentioned business challenges:

- Reducing the cost of production for commercial-scale macroalgal cultivation in open-ocean sites.
- Improving the understanding of the product(s) ingredients, and the seasonal variation of these, to optimise harvest strategies and to optimise the value of the crop.
- Reducing the cultivation cost by improving the seeding method and introducing a new valuable species to the product portfolio.

This Industrial PhD thesis describes the research performed and how the business challenges were investigated, tested and evaluated and finally supported the operational optimisation. The company Ocean Rainforest was used as a case study, though the findings can be used or evaluated more generally in the western and remaining world. The findings will help the company to make detailed product specification and documentation, find suitable product applications and specify future biorefinery perspectives. These results will in general strengthen commercial macroalgal cultivation in the Faroe Islands.

1.1 Objectives

The overall objective of this PhD study was to obtain in-depth knowledge about the biochemical composition of commercially attractive macroalgal species cultivated for sale to the food and feed market, and to optimise seeding and cultivation methods.

The research was structured into four specific objectives:

- To optimize growth rate and yield of the macroalgal species *Saccharina latissima* and *Alaria esculenta* cultivated at sea under open-ocean conditions by determining optimal growth conditions.
- To obtain data on the relationship between the chemical composition of commercially interesting target compounds and the seasonal, site and depth variation in the macroalgal species *S. latissima*, *A. esculenta* and *Laminaria digitata* cultivated in the Faroe Islands.

- To investigate optimal seeding techniques of *S. latissima* and *A. esculenta* from a commercial perspective.
- To investigate and optimise induction methods for seeding of the red macroalgal species *Palmaria palmata*, to test trigging parameters for controlled spore release and up-concentrate seeding material for commercial production.

1.2 Hypotheses

The main research hypotheses of the work were:

H1.1	Multiple partial harvesting is possible for *S. latissima* and *A. esculenta* when part of the blade is left for regrowth and the macroalgae are cultivated in a site with high water flow and stable seawater temperature.
H1.2	The growth and yield of *S. latissima* and *A. esculenta* depend on cultivation site, season, year and depths.
H1.3	The special designed MacroAlgal Cultivation Rig has an aquaculture output comparable to that of horizontal lines used in China (97.4 tonnes ww/ha/year); and spacing of growth lines and spacing of rig have a major impact on the aquaculture output.
H2.1	Seasonal variation is expected among the biochemical compounds (dry matter, ash, protein, total amino acids, lipids, total fatty acids, minerals, iodine, carbohydrates, antioxidants and vitamins) for the cultivated macroalgal species *S. latissima*, *A. esculenta* and *L. digitata*.
H2.2	Changes in growth conditions (site and depth) are expected to show a variation between the biochemical compounds (dry matter, ash, protein, total amino acids, lipids, total fatty acids, minerals, iodine, carbohydrates, antioxidants and vitamins) for the cultivated macroalgal species *S. latissima*, *A. esculenta* and *L. digitata*.
H3.1	Direct seeding method on ribbons or nylon ropes has lower cost and higher growth length compared to seeded twines nursed in a hatchery and subsequently twisted around polypropylene ropes.
H3.2	*Palmaria palmata* will release spores when exposed to low light conditions, no water flow or after freezing.

H3.3 *Palmaria palmata* is expected to seed nets when nets are deployed in a natural *P. palmata* bed, and the nets can be transplanted at sea for increased yield and possibly multiple partial harvesting without reseeding.

2 Selecting macroalgal species

Before initiation of the cultivation experiments, three target species were chosen for cultivation. These were *Alaria esculenta* (winged kelp), *Saccharina latissima* (sugar kelp) and *Palmaria palmata* (dulse) (**Figure 2.1**). This chapter describes the characteristics of these algae and why these species are relevant to cultivate.

Macroalgae are a diverse group of marine, multicellular, photosynthetic organisms that are lacking true roots, stem and leaves. Instead, macroalgae develop holdfast for attachment, stipe and blade(s) (**Figure 2.1**). All parts of an alga take up nutrients and separate cells can as such function on their own, though many species have internal transportation (Hurd et al. 2014). To grow macroalgae it requires light, CO_2, nutrients and (most often) a substrate.

In part of the macroalgae life-cycle the alga is a microscopic organism (i.e. gametophytes of *S. latissima*) and another part of the life-cycle is seen as a large organism (i.e. sporophyte of *S. latissima*) that can grow to a length of several meters and be harvested as biomass and utilised by humans. The life-cycle is explained in chapter 5 "Seeding of commercially interesting species".

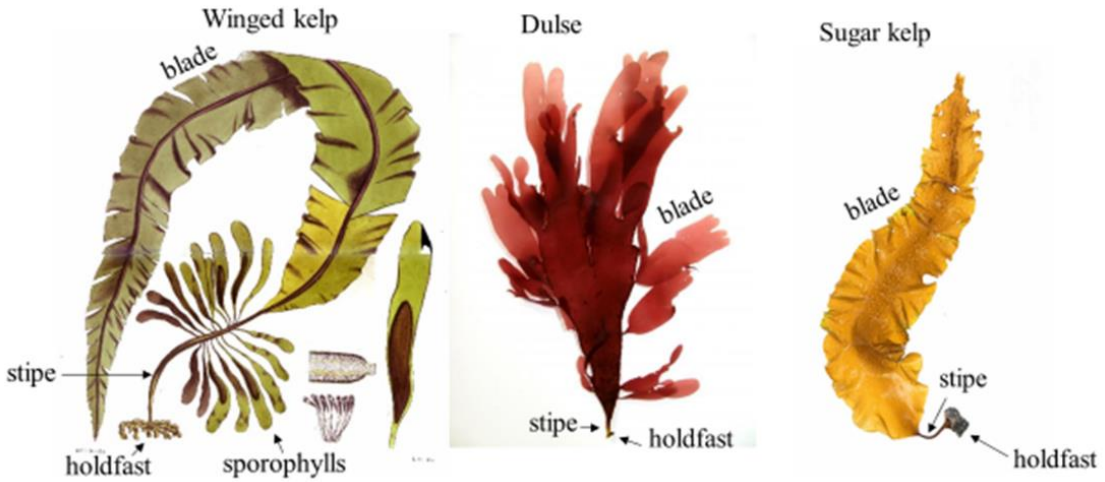


Figure 2.1 Holdfast, stipe and blade explained for three macroalgal species Winged kelp (*Alaria esculenta*), dulse (*Palmaria palmata*) and sugar kelp (*Saccharina latissima*). The pictures origins from: www.macoi.ci.uc.pt, www.seaweeds.uib.no, and www.fineartamerica.com.

The choice of macroalgal species has a high importance for a successful cultivation, and should be selected based on that the species are native and the choice should consider the hydrological

and hydrochemical conditions of the cultivation site, as the growth conditions have significant importance for the macroalgae growth. The site-specific conditions has an impact on the nutritional composition and morphology of the cultivated macroalgae (e.g. Holdt and Kraan 2011; Peteiro and Freire 2011). Finally, the cultivation method and structure should be appropriate for the species.

Macroalgal species are grouped into three phyla: Chlorophyta (green), Rhodophyta (red), and Ochrophyta (brown) and approximately 10,000 marine macroalgal species are known (Levine 2016, Makkar et al. 2016). Globally, relatively few macroalgal species (~200) have been utilised for production and 34 species are being cultivated (Buschmann et al. 2017, Ferdouse et al. 2017). Of these, only seven species count for more than 95% of the entire cultivated amount (**Figure 2.2**).

The order Laminariales includes the large brown macroalgal species often referred to as kelps. Since the mid-20-century, kelp species have successfully been cultivated in Asia because of the discovery and full understanding, and hence large-scale seeding became possible (more about seeding in chapter 5 "Seeding of commercially interesting species".

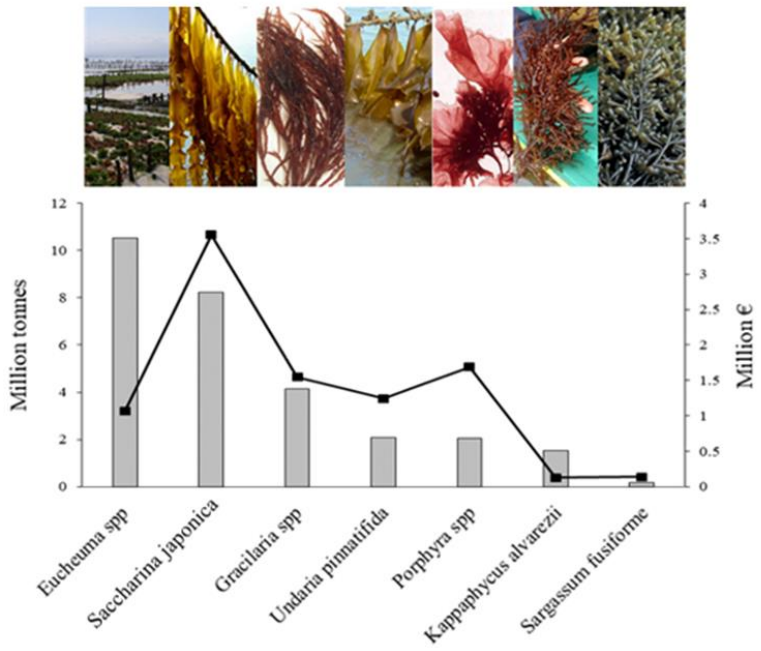


Figure 2.2 The seven macroalgal species that count for 95% of the global macroalgal cultivation. The total quantities cultivated are shown on the left axis (pillars; million tonnes ww) and the values they represent are shown on the right axis (solid line; million €). Data from 2016 (Ferdouse et al. 2017).

China is the most important producer with an annual production of 14 million tonnes ww (2015 data). Of the seven major species that are being globally cultivated, the large brown macroalgal species *Saccharina japonica* (Japanese Kombu) and *Undaria pinnatifida* (Wakame) count for 65%. *Saccharina japonica* and *U. pinnatifida* can grow to several meters and are sold as food. These species are not native in Europe, and therefore the European species most similar are an obvious choice to cultivate. Hence, the kelps *S. latissima* and *A. esculenta* have been tried cultivated for commercial utilisation.

Saccharina latissima has the common name sugar kombu or sugar kelp and is thus the Atlantic sister species to *S. japonica*. *Alaria esculenta* has the common name winged kelp and is known as the European and North Atlantic Wakame as it belongs to the same family as *U. pinnatifida* and has similar taste with a nutty flavour.

The two North Atlantic kelps *S. latissima* and *A. esculenta* were used for the commercial cultivation trials in this present work, because they both have attracted commercial interest for human consumption as sea-vegetables (Peteiro and Freire 2011, 2013a, Marinho et al. 2015a) and as an ingredient in food, feed and cosmetic products due to a promising nutritional profile (chapter 5 “The biochemical composition of cultivated macroalgae”).

Saccharina latissima grows on the lower shore in semi-exposed areas, whereas *A. esculenta* growths in highly wave-exposed areas along the shoreline. Both algae are distributed along the North Atlantic Ocean, in the Arctic areas, and along the northern Pacific coasts. *Saccharina latissima* is generally distributed in oceans with higher temperatures and/or lower salinities than *A. esculenta*, for example in the brackish sea between Sweden, Norway and Denmark, where *A. esculenta* do not grow.

Saccharina latissima is a short-lived perennial, that loose blade-biomass during winter and live up to 3-4 years (Nielsen et al. 2014). Most *A. esculenta* die or are removed by wave action during autumn, though, some may overwinter into second season. It is not known whether these individuals gets reproductive in two calendar years (Pfister 1991).

Like other kelps, both algae produce large biomass, and especially *S. latissima* is described as having good potential for commercial-scale cultivation in Europe and in North America (Wegeberg et al. 2013, van den Burg et al. 2016).

Another commercially interesting species is the red macroalgae *Palmaria palmata*, commonly known as dulse, is found in cold waters of the North Atlantic and North Pacific (Morgan and Simpson 1981). The alga is often growing under partially shaded conditions as an epiphyte on the stems of *Laminaria* spp. or on rocky coastlines in the sublittoral zone (Lüning 1990). *Palmaria palmata* is one of the few species that are consumed in the western world and the species is known for a popular flavour and high protein concentration (8-35% of dw).

Cultivation trials of *P. palmata* began when the pressure on wild populations increased due to commercial harvesting (Pang and Lüning 2004, Edwards and Dring 2011). This has intensified research activities aiming the use and cultivation of this alga, but no optimal cultivation method has been identified yet. In this work seeding methods of *P. palmata* will be investigated.

3 Macroalgal cultivation at sea

This chapter describes results found when cultivating macroalgae at sea in the Faroe Islands. To better follow the used methods and conditions, and how the cultivation is different from other initiatives, the chapter also explains the background for Asian cultivation methods when growing kelp species in large-scale.

In China, the kelps *Saccharina japonica* and *Undaria pinnatifida* are cultivated over a two-year period. A seeded substrate is placed at 1-2 meters below the surface using longlines that are held in position by floats and anchors (more details in Titlyanov et al. 2006). The cultivation activities are in the nearshore sheltered sea areas where the horizontal longlines are placed next to each other and make up huge macroalgal fields (**Figure 3.1**).

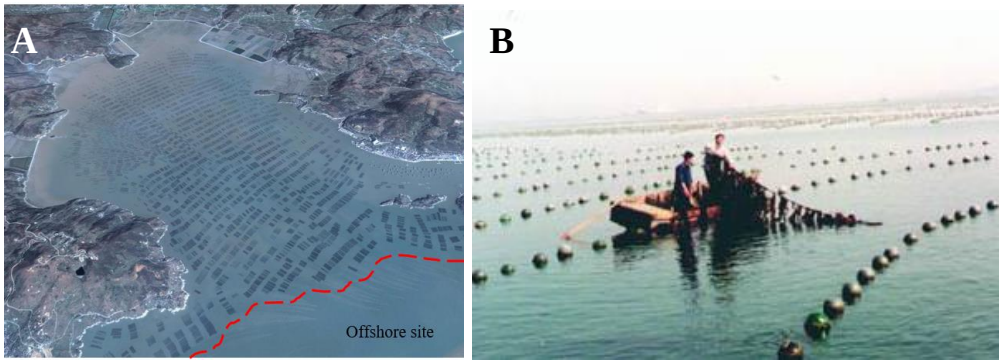


Figure 3.1 (a) Yanpu Bay in the eastern coastal province Zhejiang, China, showing large-scale macroalgal farming. The red line indicates where macroalgal framing halts due to rough sea conditions. **(b)** Kelp farming using longlines and buoys in China. (a): http://pcgladiator.blogspot.com/2009/03/seaweed-farms.html (from 2009), and (b) FAO http://www.fao.org/docrep/006/y4765e/y4765e0b.htm.

The ropes are initially seeded using pieces of seeded pieces of twine with juvenile sporophytes (the diploid life stage of the algae) that are inserted with spacing along the horizontal growth line. The seeding material is produced on land in large hatchery tanks. When harvested, the cultivation lines are dragged through a metal ring fastened in a crane to scrub-off all biomass. In some regions, macroalgal farming has been so successful that expansion is desired, though suitable cultivation sites in sheltered areas are in high demand.

The millions of tonnes produced have required artificial fertilization to maintain productivity. Regrettably, the fertilization has resulted in ecosystem disturbance and a decrease of biodiversity (Roesijadi et al. 2008).

A solution to these special and environmental problems is to produce macroalgae further off the shore where space is available, and higher water flow can provide necessary nutrient to the crop. However, the combination of the many biological, engineering and economic issues together makes open-ocean mariculture challenging, and until now, these offshore sites have not been utilised commercially (Roesijadi et al. 2008).

Like in Asia, coastal areas are highly utilised in the western world – though not for macroalgal cultivation – and new space-demanding marine industries are not well recognised. The utilisation of sheltered and nearshore sites becomes, therefore, a challenge due to spatial occupation, but also sustainability concerns.

Another challenge for a commercial macroalgal cultivation in the western world is to match the market price. Today, macroalgal biomass is sold to a low price compared to other products from aquaculture, for example fish. Furthermore, the Asian methods are labour intensive, and salary is low compared to salaries in the western world.

To solve these challenges and to help increase the global production of macroalgal biomass, low cost and optimised cultivation methods is needed to be able to use nearshore exposed and offshore sites in Asia and in the rest of the world.

This work investigated growth under open-ocean conditions for the two kelps *Alaria esculenta* and *Saccharina latissima* in the Faroe Islands. The following section was based on the findings of **PAPER I** and **PAPER II**, but also included results that have not previously been presented.

PAPER II provides an overview of former structures that have been tested in open-ocean locations. The paper evaluates their technical and economic feasibility. The conclusion of the paper is that until recently an offshore cultivation strategy has seemed very expensive and technically demanding, but due to a newly developed MACR by Ocean Rainforest and other initiatives that are trying to make offshore macroalgal cultivation a reality, the strategy seems to be reasonable and may allow considerable mass macroalgal production within foreseeable future. The MACR will be explained in detail in section 3.2.

The MACR has been optimised several times since first design and in this section the findings were presented and compared with traditional horizontal cultivation methods. Furthermore, this work investigated the optimal cultivation depth (length of vertical growth lines).

PAPER I provides growth and yield results of commercial macroalgal cultivation in the Faroe Islands using the MACR. The paper also includes cost estimations and presents results of experiments using multiple partial harvesting (**Figure 3.2**).

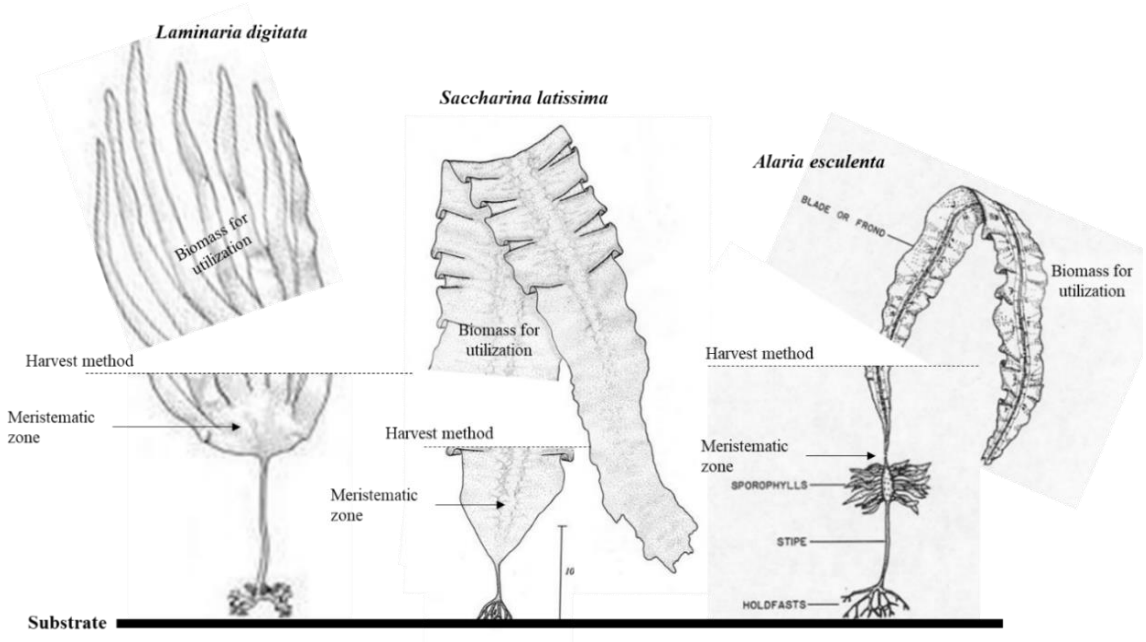


Figure 3.2 A technical drawing of a harvest method where the macroalgae are partially harvested distally to the growth zone (meristematic zone) leaving holdfast, stipe and part of blade for re-growth.

Several harvests of the macroalgal biomass without reseeding were termed "multiple partial harvesting" in **PAPER I**, and this method was suggested to increase yield and thus reduce the cost of seeding and deployment, however, was the effect of this harvesting not known.

The multiple partial harvesting method is used for red and green macroalgae but has not previously been proved for the kelps. The traditional harvesting of kelps in Asia only supports one harvest of the entire biomass due to high summer temperatures. The multiple harvesting technique has been suggested because the growth zone (meristematic zone) of the kelps is located above the stipe and below the newly grown blade. So, cutting off the old blade and leaving the meristematic blade part, the stipe and holdfast thereby allow the alga to regrow. For

A. esculenta the meristematic zone might even be at the base of the frond, adjacent to the stipe, but this has not been proved anatomically (pers. comm. Susan Brawley, University of Maine, US).

Additionally, the kelp *Laminaria digitata* was a by-crop in the second and third year of cultivation and will also be described briefly in this work.

3.1 Cultivation sites

The cultivation experiments were made in the fjord Funningsfjørður, the Faroe Islands (**Figure 3.3**). The cultivation took place at two sites: the outer part (A71) and the central part of the fjord (A02) (**Figure 3.4**).

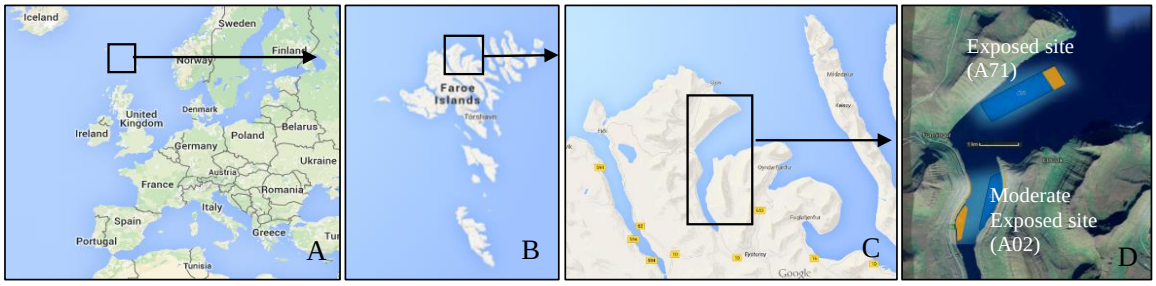


Figure 3.3 (a) A map showing Europe with a square around the Faroe Islands, **(b)** the Faroe Islands with a square around the north of Eysturoy where the cultivation took place, **(c)** the fjord Funningsfjørður, and **(d)** a satellite photo of the cultivation sites in Funningsfjørður. Orange coloured areas are the macroalgal cultivation sites and blue coloured areas are fish aquaculture. Source: ® Google maps (2018).

The site located at the outer part of the fjord (62.3030° N, 6.9267° W) was termed a "nearshore exposed site" according to a determination of cultivation sites presented in **PAPER II** (**Table 3.1)**. The nearshore exposed site had occasional significant wave heights of 6 m, the site had water current of 15–25 cm/s (Bruntse et al. 1999, Norði and Patursson 2012) and a water depth of 50-70 m. This site was exposed to oceanic-swells from northeast and a local researcher Øystein Patursson from Fiskaaling indicates that the significant wave height can be even 7-8 m (pers. comm.).

The site in the middle of the fjord (62.2677° N, 6.9591° W) was categorised a "nearshore sheltered site" according to the same determination (**Table 3.1)**. The nearshore sheltered site had occasional wave heights up to 3 m (Bruntse et al. 1999), the site was exposed to similar or

lower water current (no data exist), and a water depth of 20-30 m. Both sites were near salmon farms (less than 700 m distance).

Table 3.1 Three categories defining macroalgal cultivation sites using five site-description parameters (**PAPER II**).

Description / Categories	**Natural seaweed**	**Water depth**	**Max. wave exposure**	**Current speed**	**Distance from shore**
Offshore	No	≥ 25m	> 3m sign.	≥ 25 cm/s	> 3NM
Nearshore Exposed	No	≥ 25m	> 3m sign.	≥25 cm/s	< 3NM
Nearshore Sheltered	Yes	< 25m	< 3m sign.	< 25 cm/s	< 3NM

Figure 3.4 The nearshore exposed site **(left)**. The picture was taken in an easterly direction. The nearshore sheltered site **(right)**. The picture was taken in a south-westerly direction.

The North Atlantic Current, which originates from the warm Gulf Stream, brings warm water to the area, providing a relatively stable water temperature ranging from 7 to 11°C during the year (**Figure 3.5** and **Figure 3.7a**) (Bruntse et al. 1999, Larsen et al. 2008). The salinity is very stable at 35.0-35.2 (Bruntse et al. 1999). The nitrate concentration has been measured to be 8-12 µM during winter and spring and in most years starts to decrease in May during the spring bloom, and it decreases more in the shallow waters than offshore. Large interannual variability was observed for the timing of the decrease in nitrate concentrations as well as in the minimum level of nitrate concentrations during summer (Steingrund and Gaard 2005). Incoming light was highest during May and June and very low during winter months (**Figure 3.6**), and there was a strong decrease in light intensities (Lux) with lowered depths below sea surface (**Figure 3.7b**). Daylength had a variation of eight hours from winter to spring (**Figure 3.7c**). The attenuation of light seems to be higher in the nearshore exposed than in the nearshore exposed site.

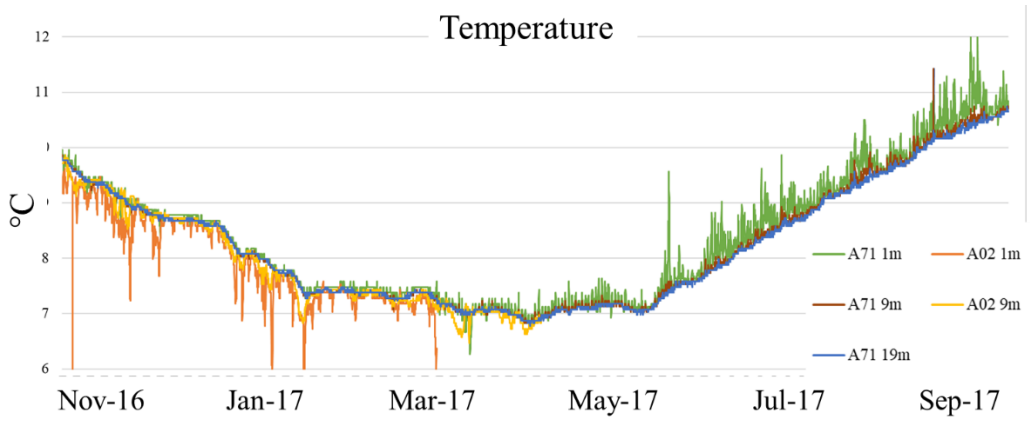


Figure 3.5 Mean temperature (°C) measured hourly by HOBO-loggers (n=2) and placed at 1, 9 and 19 m below sea level from Nov. 2016 – Nov. 2017 at the nearshore exposed site (A71) and the nearshore sheltered site (A02) in Funningsfjørðiur, Faroe Islands. The Nearshore sheltered HOBO-loggers were removed in April 2017. Another season (Nov. 2017-May 2018) was monitored having the same temperature pattern as the first year (data not shown).

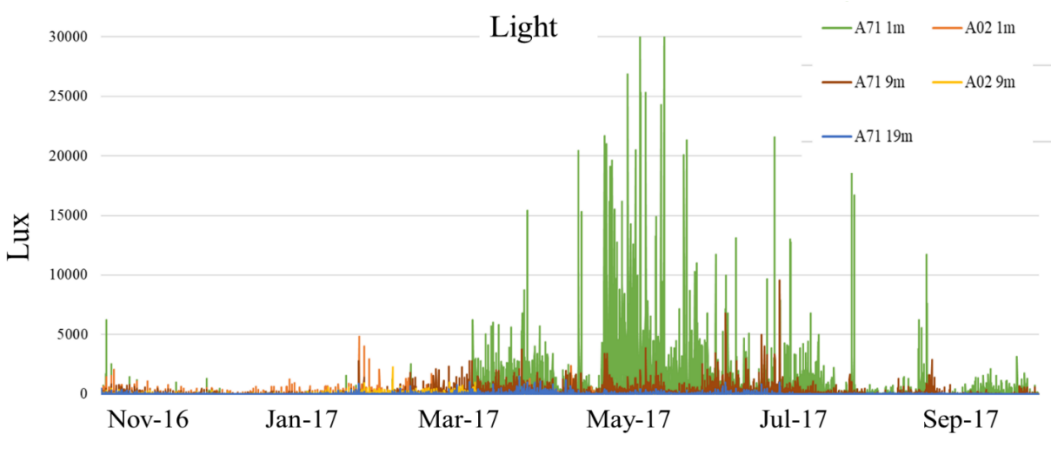


Figure 3.6 Light (Lux) measured hourly by HOBO-loggers (n=2) and placed at 1, 9 and 19 m below sea level from Nov. 2016 – Nov. 2017 at the nearshore exposed site (A71) and the nearshore sheltered site (A02) in Funningsfjørðiur, Faroe Islands. The Nearshore sheltered HOBO-loggers were removed in April 2017. Another season (Nov. 2017-May 2018) was monitored having the same temperature pattern as the first year (data not shown).

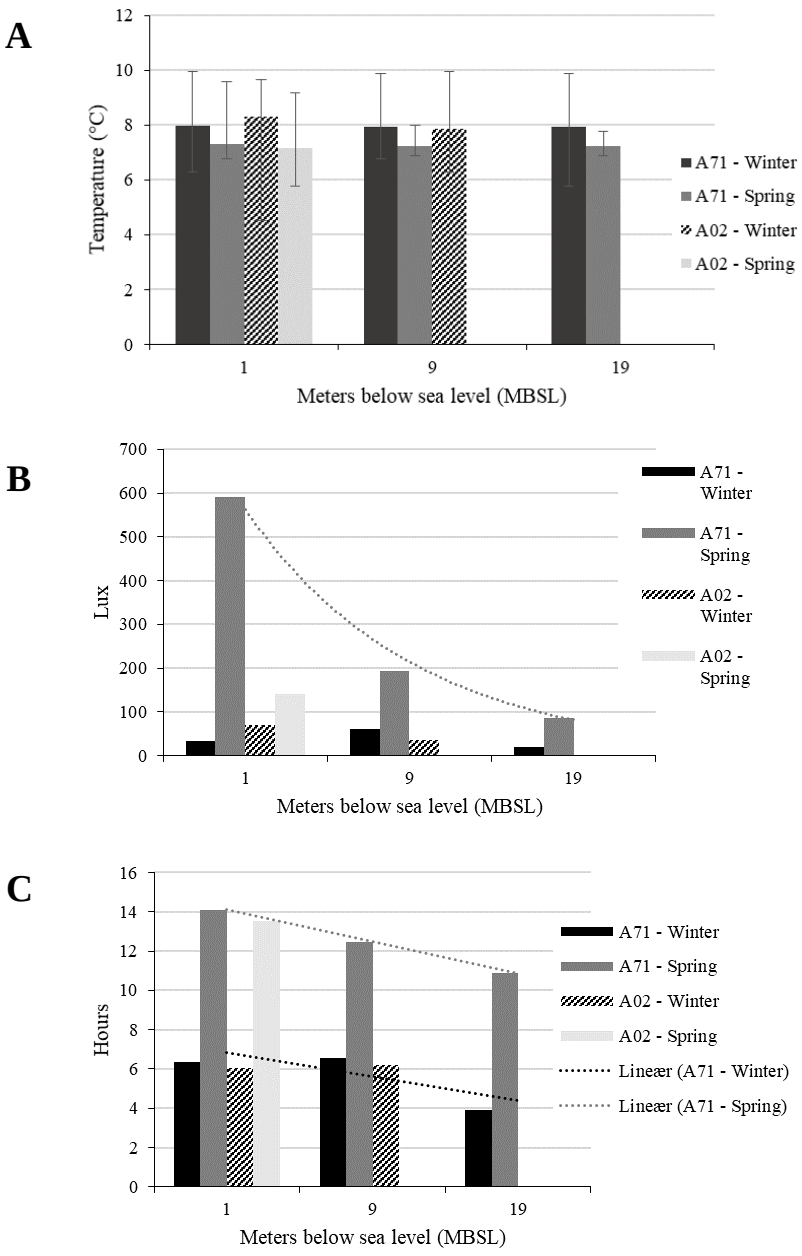


Figure 3.7 (a) Mean±SD temperature (°C), **(b)** light intensity (lux) and exponential trend line from spring ($y=1476e^{-0.97x}$), and **(c)** daylength in hours including the linear trend line from winter (y= -1.2x+8.1) and spring (y= -1.6x+15.7) at 1, 9 and 19 m below sea level during winter (Nov 2016 - March 2017) and spring (March 2017 - May 2017) at the nearshore exposed (A71) and the nearshore sheltered (A02) site in Funningsfjørður, Faroe Islands, using HOBO-loggers (n=2).

3.2 Method and materials

3.2.1 Cultivation method and optimisation

The MacroAlgal Cultivation Rig (MACR; **Figure 3.8**) was designed by Ocean Rainforest in 2008 for offshore macroalgal cultivation (**PAPER I**). In brief, the design consists of a fixed line horizontal suspended below sea level. The length of the fixed line can be up to 600 m and is held in position at 10 m below sea level (MBSL). Both length of fix line and position below sea level can be adjusted after the conditions of the site or the type of macroalgae cultivated. Two main surface floats are connected to the fixed line and were submerged in a static state. The mooring system consisted of four anchors. From the fixed line, vertical growth lines are attached with a float fixed at the opposite end stretching the lines in a vertical position. The growth lines are intended to bend away from high energy zones during bad weather, and the design of the structure mimics *Laminaria hyperborea* stems that move with currents and waves and are attached on the sea bottom where less wave power occurs.

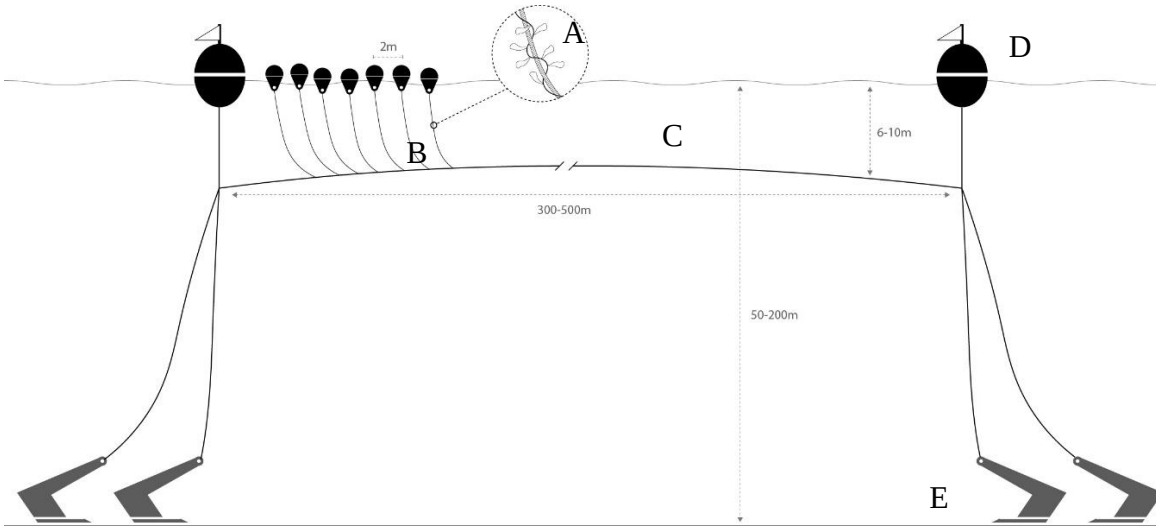


Figure 3.8 Schematic drawing of the Macroalgal Cultivation Rig (MACR) constructed by Ocean Rainforest Sp/F and published by ©Elsevier. The construction can be deployed for macroalgal cultivation at wave-exposed sites with a water depth of 50–200 m. Seed lines (A) are twined around growth lines (B) that are attached at 2 m intervals to the fixed line (C) by a loop and held in a vertical position by a buoy. Two main surface floats (D) and four steel anchors (E) ensure the right position of the rig. A copy of figure 2, **PAPER I.**

The MACR has proven to be functioning since 2010 in the nearshore exposed site. The rig has been deployed more than 10 times and all structures have survived with no loss of equipment. Beside of these promising results, continued investigation was warranted to solve some of the remaining technical and economic challenges of the operation in the Faroe Islands**.**

The MACR was the first in a serious of MACR's using same basic principles but with modifications to different cultivation sites. The modified MACR's were tested as a part of this work.

The first MACR, described above, was named RUNI (**Figure 3.8**). Pilot tests using the MACR-RUNI at the nearshore exposed site with 6 m long vertically growth lines showed high yield even in the lowest meters. Therefore, 10 m long growth lines were tested from 2014-2016 (**PAPER I**). The new results showed that growth migth be possible even further down. Consequently, some of the growth lines were deployed from the fixed line and downwards providing a total cultivation length from the surface and 20 m down (**Figure 3.9**).

MACR-RUNI did not work optimally in the nearshore sheltered site as current speed were too weak or had a wrong orientation thus tangling of lines occurred. Consequently, two other cultivation rigs were designed. These two rigs were designed for sites with less wave and current action, than the MACR-RUNI.

MACR-URD was designed to hold a high capacity of *A. esculenta* in the top three meters below sea level. Hence, 3 m long lines in sections of 10 with spacing of 0.5 m were attached using a sink-rope. The rig was tested from 2017 at the nearshore sheltered site.

MACR-DIA was designed for *S. latissima,* which can grow deeper than *A. esculenta.* The rig and the vertical growth lines stand in a stable position and allow the growth lines to be placed very close thereby increasing the capacity. The rig was also tested since 2017 at the nearshore sheltered site.

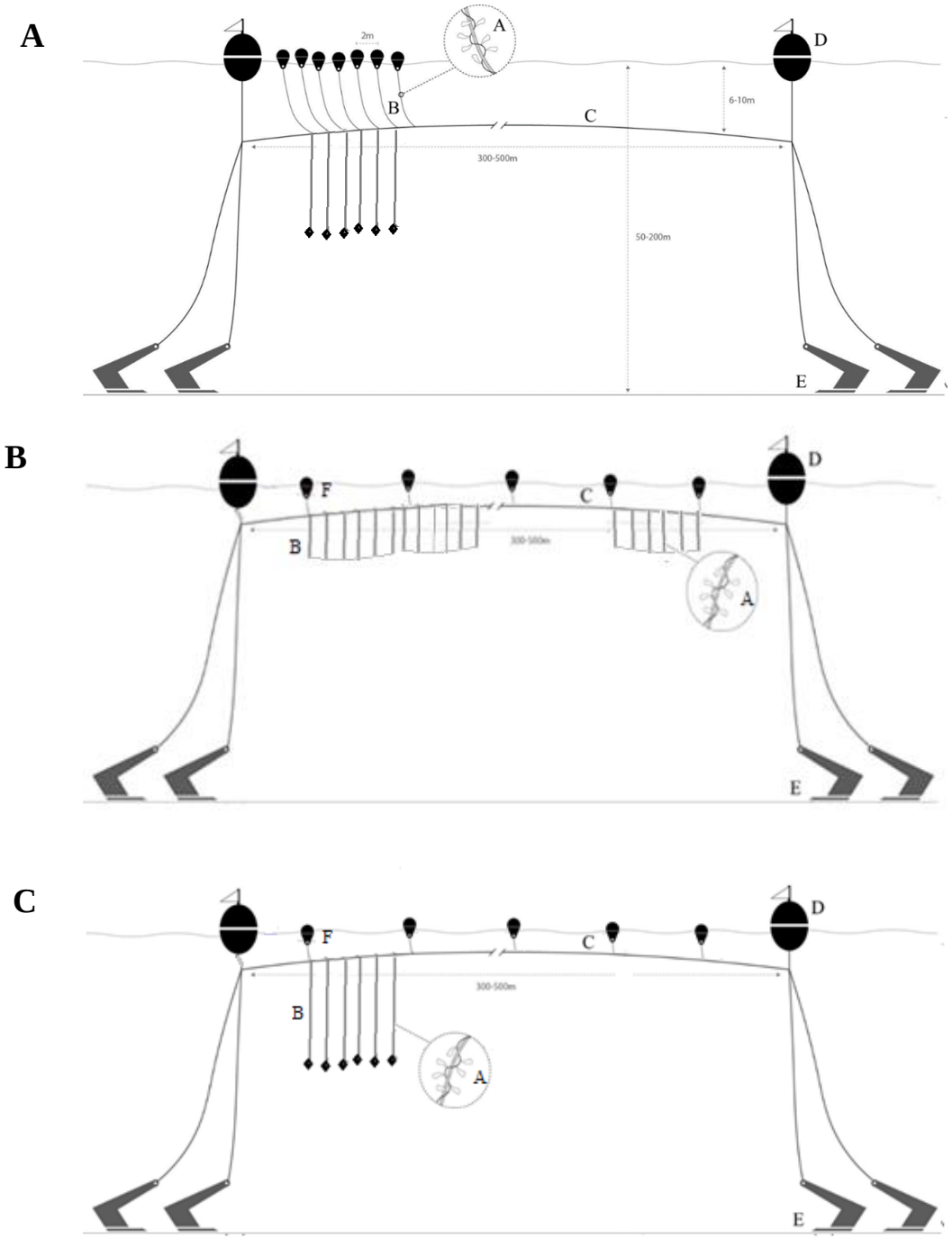


Figure 3.9 Modifications of the Macroalgal Cultivation Rig (MACR) constructed by Ocean Rainforest Sp/F. **(a) MACR-RUNI** with double capacity, having 10-meter-long growth lines attached to the fixed line and a sink attached to the free end of the rope, thus hanging vertically from 0-20 meters below sea level. **(b) MACR-URD** optimised for macroalgal cultivation at nearshore sheltered sites and suitable for deployment at 20-50 m water depth. Growth lines (B) are attached at 0.5 m intervals to the fixed line (C) and held in a vertical position by a sinking line holding down 10 vertical growth lines. The fixed line has smaller buoys to hold it in position at 50 cm below the sea surface. **(c) MACR-DIA** designed for nearshore sheltered sites. With a fixed line (C) suspended horizontally at 1 m depth. From the fixed line 7-8 m long growth lines are attached with 1 meter spacing (B), and a sink (0.5 kg) stretching the lines in a vertical position. Full explanation off all letters in

In total, 10 rigs were deployed from 2010 until 2019 (**Table 3.2**). In the PhD work period, nine rigs were seeded and deployed. A total of 43.4 km of growth lines was deployed and three versions of the MACR were tested. From 2017, all deployed lines were seeded using a direct seeding method (chapter 5 "Seeding of commercially interesting species".

Table 3.2 List of macroalgal lines deployed in Funningsfjørður, the Faroe Islands. MACR = MacroAlgal Cultivation Rig, SL = *Saccharina latissima,* LH = *Laminaria hyperborea,* AE = *Alaria esculenta,* PP = polypropylene, hatchery = twine line with juvenile sporophytes nursed in the hatchery (~6 weeks) and twined around a rope before deployed at sea.

SITE	MACR	MACR version	Deployment (date)	Species	Capacity (km)	Distance between growth lines (m)	Growth line length (m)	Substrate	Seeding method	Times harvested	Removed/ changed
Nearshore exposed site (A71)	0	RUNI	Mar. 2010	SL/LH	0.9	2	6	PP	hatchery	0	Sep. 2016
	1	RUNI	Nov. 2014	SL	2.3	2	10	PP	hatchery	5	Nov. 2016
	2	RUNI	Nov. 2014	SL/AE	2.3	2	10	PP	hatchery	4	Nov. 2016
	3	RUNI	Dec. 2015	SL	2.6	2	10	PP	hatchery	2	Nov. 2016
	4	RUNI	Mar. 2016	AE/SL	2.3	2	10	ribbon/PP	lirect/hatchery	2	
	1 (new)	RUNI	Oct. 2016	AE	2.3	2	20	PP	hatchery	2	
	2 (new)	RUNI	Jan. 2017	SL/AE	2.9	2	20	ribbon	direct	1	
	3 (new)	RUNI	Feb. 2017	AE/SL	3.8	2	20	ribbon	direct	1	
Nearshore sheltered site (A02)	1	RUNI	Mar. 2015	SL/AE	2.5	2	10	PP	hatchery	3	Oct. 2017
	2	RUNI	Mar. 2015	SL/AE	2.5	2	10	PP	hatchery	2	Oct. 2017
	3	URD	Oct. 2017	AE	3	0.5	3	nylon	direct	1	
	4	URD	Oct. 2017	AE	3	0.5	3	nylon	direct	1	
	5	DIA	Jan. 2017	SL/AE	8	-	8	nylon	direct	1	
	1 (new)	DIA	Oct. 2017	SL	1.8	2.2	8	nylon	direct	2	
	2 (new)	DIA	Oct. 2017	SL	3.2	1.8/0.9	8	nylon	direct	1	
Total	**15**				**43.4**						

3.2.2 Seeding and deployment method

The optimisation of the seeding method is explained in chapter 5 "Seeding of commercially interesting species". Here, the standard method used by Ocean Rainforest is explained. For further details see **PAPER I.**

Seeding material was produced by the company Hortimare BV, Netherlands, using a standard procedure for kelp sporulation. Fertile *S. latissima* and *A. esculenta* were collected from wild populations in Funningsfjørður during winter approximately a year before planned deployment. From the sterilized fertile sori, spore release was done by leaving the sori dehydrating and in darkness until next day. The spores were released to sterile and filtrated seawater and the gametophytes were nursed until enough biomass was reached. Next step was then an induce fertility using white light, where the gametophytes developed into juvenile sporophytes within two weeks (size<0.1 mm). The density of the seeding material was approximately 200

sporophytes/m. The juvenile sporophytes were seeded on twine (2 mm) using a binder-mixture, twined around coils and cultivated in hatchery tanks for a one month period before deployment (size <1 mm).

The juvenile sporophytes were deployed at sea in autumn. A day before deployment, the seed lines were twined around a growth line of 14 mm polypropylene three strained twisted rope. These growth lines had a length of 10 m and were attached along the fixed line with spacing and each line had a buoy attached in the opposite end to provide uplift.

3.2.3 Commercial harvest method

The harvest method and yield monitoring can be found in **PAPER I**. Here an expanded description is provided.

The harvest method was manual cutting with a knife. Only the blades were cut off; leaving holdfast, stem and part of the blade (**Figure 3.2** and **Figure 3.10b**). The optimal cutting length of *A. esculenta* and *S. latissima* has not been described previously, because the multiple partial harvesting method has not been tested for these species before. The cutting method was therefore investigated and described in **PAPER I**. Cutting distally to the sporophylls, leaving 5–15 cm of the blade, entire holdfast, stem and sporophylls, was found to be optimal for *A. esculenta* and for *S. latissima*. This cutting length was used to ensure preservation of the meristematic zone to allow re-growth.

On a typical harvesting day, three staff members operated the vessel: one captain and two deckhands. From the harbour, there was a one-hour sailing distance to the nearshore exposed site and a half-hour sailing to reach the nearshore sheltered site. When reaching the site, the fixed line was lifted using a crane, and when lifted, the fixed line was attached to two pulley blocks, located on starboard at the bow and at the stern (**Figure 3.10a**).

The buoys were kept downwind, drifting away from the boat, to keep them from tangling. Under optimal conditions there was a current across the fixed line. If the current was weak, the buoys would not drift downwind and instead tangle when the fixed line was lifted to the side of the boat.

The staff manually caught a top buoy with a long-hooked stick, and retrieve it, until a buoy was next to the boat and the crane lifted the growth line. Once the line was on board, the crane places it over a frame of plastic tubs providing a working height around 180 cm. A staff deckhand then cut of biomass into boxes of 660L capacity (**Figure 3.10b**). Each box could hold approximately 70 kg ww of harvested macroalgae. The boxes were filled with seawater as soon as they were full for best preservation. After filled with seawater, the boxes were sealed with a lid to avoid sunlight heating the freshly harvested macroalgae and to comply with regulations of food grade raw material on land during the transportation (**Figure 3.10c**).

When the vessel arrived in the harbour, the tubs were drained of seawater before landing to facilitate easy transportation and to prevent disease to spread, complying with Faroese regulations. Transport time was a half hour by car (**Figure 3.10d**).

Figure 3.10 (a) The fixed line was attached to two pulley blocks, located on starboard at the bow and at the stern, **(b)** the growth line was placed on a frame holding the line above boxes allowing the biomass to fall into the boxes when cutting, **(c)** boxes with freshly harvested macroalgae were filled with seawater and covered by a lid before sailing to land, and **(d)** transportation from Funningsfjørður to Kaldbak for further processing.

If frozen biomass was requested, it was packed in boxes of 10 kg ww and immediately frozen in a chest freezer at -35-40 °C, hereafter stored at -20 °C. If dry biomass was requested, it was dried at 28-35 degrees, milled into smaller sizes and packed in bags or boxes according to size categories (e.g. <2mm, 2-4mm, etc.). The storage stable biomass was measured by weight before it was stored awaiting transportation to customers.

3.2.4 Registration of yield and monitoring of growth and density

The harvested and packed macroalgal biomass was used to describe yield (**PAPER I**).

Length and density were monitored monthly over a period of two years (2015-2016) for *S. latissima* and *A. esculenta* that were deployed on two MACR's in November 2014 at the nearshore exposed site and deployed on two MACR's in March 2015 at the nearshore sheltered site (**Table 3.2**). For each monitoring, three replicate lines were used. The line density (number of plants) and the six longest individuals on a meter were noted for every second meter of the vertical cultivation rope (10 m long ropes). Density above 100 visible individuals per meter was registered as >100 (**Figure 3.11**).

Figure 3.11 Field measurements of a 4.1 m long *Saccharina latissima* measured from tip of the blade to rope.

The aquaculture output was calculated according to **PAPER I** and further elaborated in **PAPER II**.

$$AO = Y/A = \text{kg dw/ha/year}$$

where AO is the aquaculture output, found from the productivity (Y) per year of a cultivation area (A) including the handling space.

3.2.5 Growth rate

The relative growth rate (RGR), which is the growth rate relative to size, is for macroalgae expected to be exponential: $y = a(1+r)^x$, where *a* is initial value and *r* is the growth or decay rate (most often represented as a percentage and expressed as a decimal).

RGR was calculated using length measurements of the cultivated macroalgae using the mean length of the six longest individuals on a meter.

The relative growth rate has the unit% increase per day and has the formula:

$$RGR = (lnL_2 - lnL_1) / (t_2 - t_1)$$

where *ln* is natural logarithm, t_1 was the initial time (in days), t_2 was time two (in days), L_1 was the algal length at t_1 (in cm), and L_2 was the algal length at t_2 (in cm).

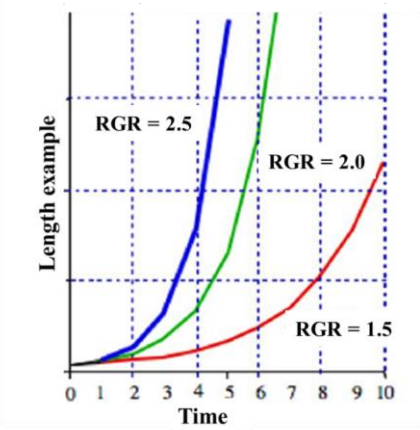


Figure 3.12
Illustration of exponential growth with time on the x-axis and here length on the y-axis. Three growth rates are shown as increased length% per day with rates of 2.5, 2.0 and 1.5, respectively.

3.2.6 Statistical treatment

Data treatment of length and density measurement included removal of all values of zero, except when within three months after deployment as zero is a normal result during initial growth measurements or during winter, whereas *A. esculenta* had no biomass. In all other cases, zero numbers represented human errors from handling and are not representative for the growth potential.

Data are expressed as mean ± standard error (SE), or mean ± standard deviation (SD), stated for each figure.

The statistical treatment of the data was made in PRIMER 7. First, data were tested for normality using PERMDISP test, which is a distance-based test for homogeneity of variance using Euclidean distance. For not normally distributed data, a transformation was used to gain equally distributed data ($p > 0.05$).

Secondly, a permutational ANOVA (PERMANOVA) (one, two or three factors) was used to test the effect of year, time, site and depth on various response parameters: length, yield, density, etc. Whenever a significant difference between means or interaction of factors was revealed, a pairwise comparison among levels of factors was performed to compare the influence of parameters. Means were considered significantly different when levels of $p<0.05$ were obtained.

3.3 Results and discussion

The section is based on the work presented in **PAPER I** and **PAPER II** and additional experimental work that has not been presented previously.

3.3.1 Multiple partial harvesting results

This work has for the first time shown that multiple partial harvests are possible for the large brown macroalgae *S. latissima* and *A. esculenta* when cultivated in the Faroe Islands (**PAPER I**). From a total of 5 km of growth lines deployed in November 2014, *S. latissima* was harvested four times with a spring and summer harvest every year. *Alaria esculenta* was harvested one time the first year and two times the second year. The regrowth of *A. esculenta* was indeed surprising as the plants had no sign of being active in the months from November to March, which has also been described by Pfister (1991). The experiment showed on the other side that *A. esculenta* have a lower ability to re-grow as rapidly as *S. latissima.* After cutting *S. latissima,* the regrowth occurred from the meristematic zone at the base of the blade. For *A. esculenta* the regrowth occurred directly from the top of the stipe, or sometimes like for *S. latissima* from the blade base **Figure 3.13**. As only the stipe was visible during winter and as the spring growth was seen directly from the stipe – first as a small curly dwarf frond but later well developing into a blade - one could assume that the meristem is at the base of the frond, adjacent to the stipe. That the meristem is basal as like all other kelps, and even tough blade erode to the stipe it has most likely still a margin of basal blade, even if not visible.

Figure 3.13 *Alaria esculenta* **(left)** and *Saccharina latissima* **(right)** monitored in September after partial harvesting in July. The pruning cut was still visible at the tip of the blade indicating no degradation of the blade since latest harvested.

Experiments with cutting length of *A. esculenta,* however, proved that cutting proximally to the sporophylls, only leaving holdfast and part of stipe, killed the algae; whereas, cutting distally to the sporophylls kept them alive for regrowth in spring (**PAPER I**). This result emphasises that the meristematic zone of *A. esculenta* is distally to the sporophylls.

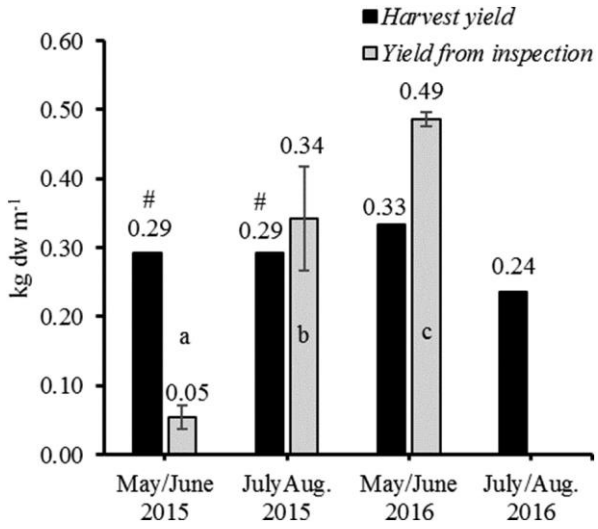


Figure 3.14 Figure is from **PAPER I**. Mean yield of *Saccharina latissima* in dry weight of one-meter growth line, published by © Elsevier. The biomass was deployed at the nearshore exposed site in the Faroe Islands in November 2014, and multiple partial harvested four times without reseeding of growth lines. Yield per meter was determined either by harvested biomass divided by the number of line meters that was harvested (black) or by average yield monitored on field inspections (replicate lines of 10 m length, n=5). Error bars represent standard deviation. Statistical different harvest yields are marked with different letters. Pillars marked with # were calculated from the total 2015-yield and not for each harvest. The yield represents biomass ready for sale and discarded biomass and biomass left for re-growth were not included.

The harvest yield was significantly different between the first, second, third and fourth harvest based on field measurements and yield registrations of harvested biomass (**Figure 3.14**).

The total yield of biomass grown in the commercial operation was challenging to evaluate because the commercial data represent biomass ready for sale, and not the biomass stock on the lines. Discarded biomass during processing and biomass left for re-growth was therefore not included. This represents an error regarding the cultivation capacity and the comparison with other yields-data; but, a very good evaluation of quantities available for sale.

In total 10 MACR's were deployed over an eight-year period, and 5 of these redeployed. In total 43.4 km growth lines were cultivated in that period, and each rig was harvested one to five times (**Table 3.2**) and a total yield of 9 tonnes dw has been registered as harvested and sold biomass over the past four years (**Table 3.3**), though the potential of the cultivation capacity was higher.

Table 3.3 Extension of **Table 3.2** providing an overview of harvested biomass during a four-year period shown as: lines harvested, total yield (kg dw), yield per meter growth line as dry weight (kg dw/m) and yield per meter growth line in fresh weight (kg ww/m). MACR = MacroAlgal Cultivation Rig, N/A = not specified, SL = *Saccharina latissima*, AE = *Alaria esculenta*, B = boxes. Red numbers were estimated from the "not specified" yield using the number of boxes filled with an average of 70 kg ww in each.

SITE	MACR	MACR version	Deployment (date)	Species	Capacity (km)	Times Harvested	2015				2016				2017				2018				Total	
							lines	kg dw	kg dw/m	kg ww/m	lines	kg dw	kg dw/m	kg ww/m	lines	kg dw	kg dw/m	kg ww/m	lines	kg dw	kg dw/m	kg ww/m	kg dw	kg dw/harvest
Nearshore sheltered site (A02)	1	RUNI	Nov. 2014	SL	2.3	5	231	980	0.4	4.2	180	679	0.4	3.8	60	67.5	0.1	1.1	Removed				1693	339
	2	RUNI	Nov. 2014	SL/AE	2.3	4	208	882	0.4	4.2	139	999	0.7	7.2					Removed				1882	470
	3	RUNI	Dec. 2015	SL	2.6	2	year of deployment								214	459	0.2	2.1					230	115
	4	RUNI	Mar. 2016	AE/SL	2.3	2					year of deployment												230	115
	1 (new)	RUNI	Oct. 2016	AE	2.3	2					year of deployment				140	54	0.0	0.4	137	275	0.2	2.0	54	27
	2 (new)	RUNI	Jan. 2017	SL/AE	2.9	1									year of deployment									
	3 (new)	RUNI	Feb. 2017	AE/SL	3.8	1									year of deployment									
	1	RUNI	Mar. 2015	SL/AE	2.5	3	year of deployment				248	601	0.2	2.4	197	459	0.2	2.3					760	253
	2	RUNI	Mar. 2015	SL/AE	2.5	2	year of deployment								Removed								601	301
	3	URD	Oct. 2017	AE	3	1									year of deployment				B:50	446			446	446
	4	URD	Oct. 2017	AE	3	1									year of deployment				B:10	89			89	89
	5	DIA	Jan. 2017	SL/AE	8	1									year of deployment									
	1 (new)	DIA	Oct. 2017	SL	1.8	2									year of deployment				214	435	0.25	2.5	435	218
	2 (new)	DIA	Oct. 2017	SL	3.2	1									year of deployment				329	336	0.13	1.3	336	336
N/A																2421				810			3231	
Total	**15**				**42.5**	**28**	439	1862	0.4	4.2	586	2280	0.4	4.5	611	3461	0.12	1.5	680	1581	0.2	1.9	9183	328

The number of total harvested lines increased from 2015 to 2018, but the total yield only increased from 2015 to 2017. The year 2018 had lower total yield than the previous years due to several factors. The lower yield, even with more lines deployed, was a direct consequence of testing new macroalgal cultivation rigs and a high seeding of *A. esculenta* in the nearshore sheltered site, where it did not have its optimal growth conditions. The year 2018 was also influenced by the fact that licences for cultivation at the nearshore exposed site was up for re-evaluation and new deployments were not accepted until a final licencing. Furthermore, the

year 2018 was the year where Ocean Rainforest installed their own drying facility, and this was a limiting factor for exploiting the full potential AO. All these issues are factors that a commercial macroalgal farm also must operate within and deal with.

3.3.2 Seasonal growth and species variation

The length of *S. latissima* and *A. esculenta* was monitored monthly over a two-year period to identify the growth pattern of the two species and also at the two cultivation sites (**Figure 3.15**). When comparing growth pattern of the two sites deployment time must be considered as the lines were deployed in November 2014 at the nearshore exposed site and in March 2015 at the nearshore sheltered site.

Alaria esculenta was harvested one time per year. The average length when harvested was 200 cm. *Saccharina latissima* was harvested four times within two years at the exposed site and two times at the nearshore sheltered site. The average length for *S. latissima* when harvested was 100, 120 and 160 cm from first, second and third harvest. The average length at the fourth harvest was not monitored (**Figure 3.15**).

The relative growth rate (RGR) was calculated for relevant time periods and presented in **Table 3.4**. An example of how the RGR's were found are shown in **Figure 3.16**. The time periods were determined after the growth pattern observed from length results (**Figure 3.15**) and a separate time period was analysed after either "a harvest" or when the length changed markedly.

The growth of was highest in spring the first year after deployment for all species and sites, though *A. esculenta* had a 0.5% slower biomass increase. Even though all experiments had all most similar RGR in spring period, the biomass on the nearshore sheltered site was not enough for harvesting (the length was less than 40 cm in June; **Figure 3.15**). Hence, the later deployment in the nearshore sheltered site was very significant for the harvest possibilities.

In the time period "summer and autumn", *A. esculenta* had a slightly declining growth rate. This result show that the species had no change of re-growing right after the multiple partial harvesting. Therefore, one could argue that the exponential growth was inappropriately stopped, and postponed harvesting may be suggested. However, the grassing pressure of *A. esculenta* increased significantly during July and the biomass was reduced by approximately 75% within

few weeks in 2017 (pers. observation). Therefore, the exponential growth rate was inhibited anyway, and harvesting must occur before grassing increase dramatically (**Figure 3.18**).

In contrast, *S. latissima* continued to grow during autumn though at a lower rate than during spring period. The harvesting of *S. latissima* at the nearshore exposed site seems to have a positive effect as RGR is higher than at the nearshore sheltered site. Though, this could also be due to a lower water transmittance or lack of nutrients in the more inshore cultivation site.

During winter the length of *A. esculenta* goes to zero, and *S. latissima* also decrease in biomass at the nearshore exposed site. Surprisingly, the growth rate for *S. latissima* at the nearshore sheltered site stays relatively high during winter. Maybe a result of accumulated carbohydrates (energy storage) for biomass growth during winter in the low-light season.

In spring the second year after deployment, the growth rate of *A. esculenta* was very high and considerably higher than for *S. latissima.* This result is probably related to the initial biomass on the lines when spring growth take off, as *A. esculenta* grows from zero to 200cm length from March to mid-May and *S. latissima* in the same period start at 70 cm length and the increased growth is therefore length but also mainly weight and building wider and thicker blade tissue. For optimal monitoring of the growth the specific growth rate must be studied.

The relative growth rate (RGR) and the specific growth rate (SGR) of macroalgae have been investigated by many. Fortes and Lüning (1980) investigated the SGR of several red, green and brown macroalgal species from Helgoland, Germany, over a growth period of one week. Also, *S. latissima* was included in the study. *Saccharina latissima* had significant higher SGR (6-9% per day when grown in 5-15 °C seawater) than found in this present study. Marinho et al. (2015b) cultivated *S. latissima* in the inner Danish waters and calculated the SGR at a site next to fish aquaculture and at a reference site, and in the period from deployment in November until harvest in May they found SGR of 1.2 and 0.9% per day at the site next to fish aquaculture and reference site, respectively. These growth rates correspond to the RGR found in the Faroe Islands, though lower but also measuring a longer lag phase from November to approximately March, which was not included in **Table 3.4**. When calculating a similar growth rate from November to May (200 days) in the Faroe Islands, the RGR was 2.3% per day. Still, markedly higher.

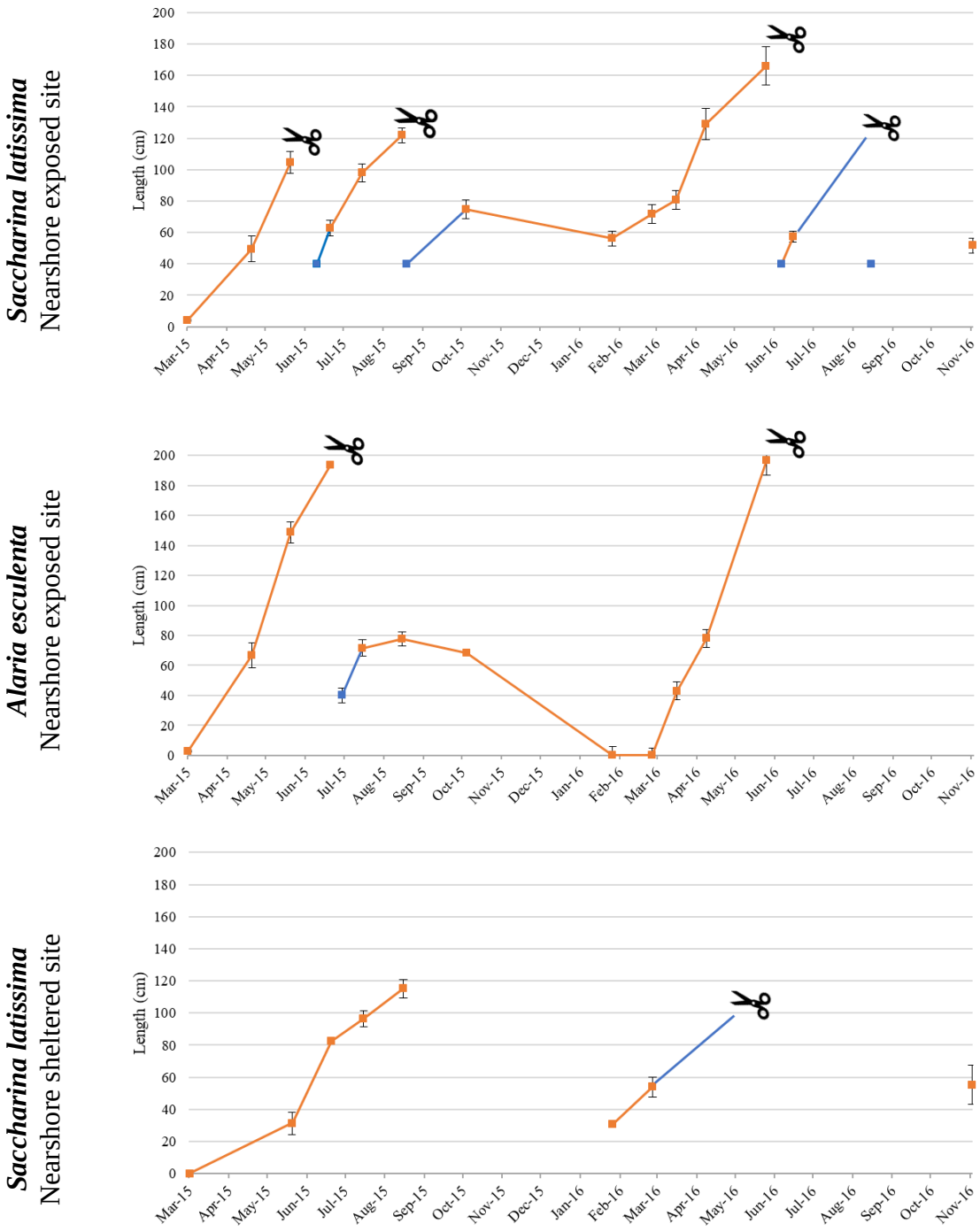


Figure 3.15 The mean maximal length (cm) ± standard error (SE) for *Saccharina latissima* and *Alaria esculenta* (n=3) seeded on 10 m long vertical growth lines and deployed at the nearshore exposed and at the nearshore sheltered site in November 2014 and March 2014, respectively. The length was monitored monthly (orange marks). The growth lines were partially harvested up to four times; harvest was indicated by scissors. The biomass left on the lines was assumed to be 40 cm long (blue marks). Data for *S. latissima* in the nearshore exposed site is a copy of figure 5 in **PAPER I**.

Table 3.4 The relative growth rate (% increase per day) for *Saccharina latissima* and *Alaria esculenta* when cultivated at the nearshore exposed or the nearshore sheltered site. Note that the time periods are not identical. n = number of measurements included in the RGR calculation. A harvest was indicated by scissors.

Species and site		Spring 2015	Summer and autumn 2015	Winter	Spring2016
Saccharina latissima Nearshore exposed site	***RGR (d^{-1})***	**4.15** ✂	**1.17** ✂	**-0.03**	**0.99** ✂
	R^2	1.0	0.9	(1.0, n=2)	0.9
	Time period	1/3-20/5	20/6-15/8	4/10-25/1	26/2-25/5
Alaria esculenta Nearshore exposed site	***RGR (d^{-1})***	**3.85** ✂	**-0.00**	**0.00**	**2.14** ✂
	R^2	0.9	0.2	(1.0, n=2)	1.0
	Time period	1/3-20/6	15/7-4/10	26/1-26/2	16/3-25/5
Saccharina latissima Nearshore sheltered site	***RGR (d^{-1})***	**4.04**	**0.59**	**1.80**	**-** ✂
	R^2	1.0	1.0	(1.0, n=2)	-
	Time period	1/3-20/6	20/6-15/8	26/1-26/2	

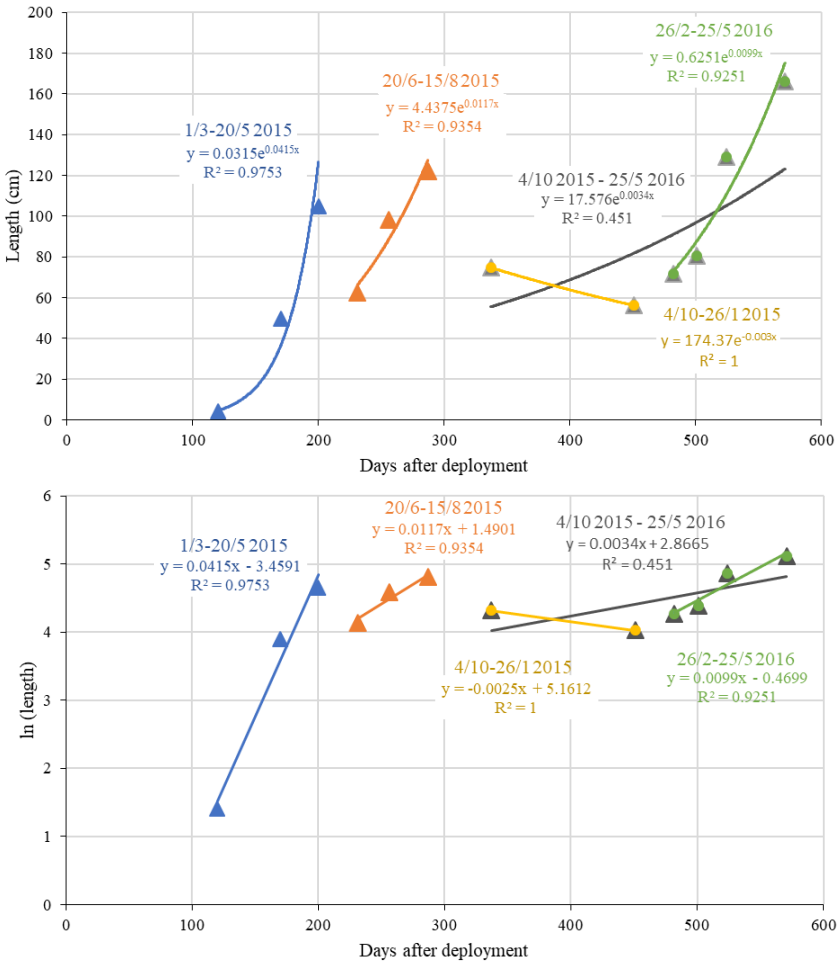


Figure 3.16 An example of how relatively growth rate were calculated for *Saccharina latissima* when cultivated at the nearshore exposed site. **(a)** The length measurements are shown at the y-axis and the days after growth lines were deployed are shown on the x-axis. Growth rates are expressed as an exponential trend line ($y = a*e^{rx}$, where r is the rate and a is the initial value). **(b)** The natural logarithm of the length is shown at the y-axis and the days after growth lines were deployed are shown on the x-axis. Growth rates are expressed as a linear trend line ($y = ax + r$, where is the rate and a is the initial value).

For an isolated evaluation of the results, the interaction of time and species was investigated and lengths of the macroalgae were monitored in May, June and January for both species. Again,

the result was a faster growth of *A. esculenta* during spring and summer, but with no growth/biomass during winter months. There was therefore also a significant effect of the interaction of species and time and the differences are shown in **Figure 3.17** (p=0.001).

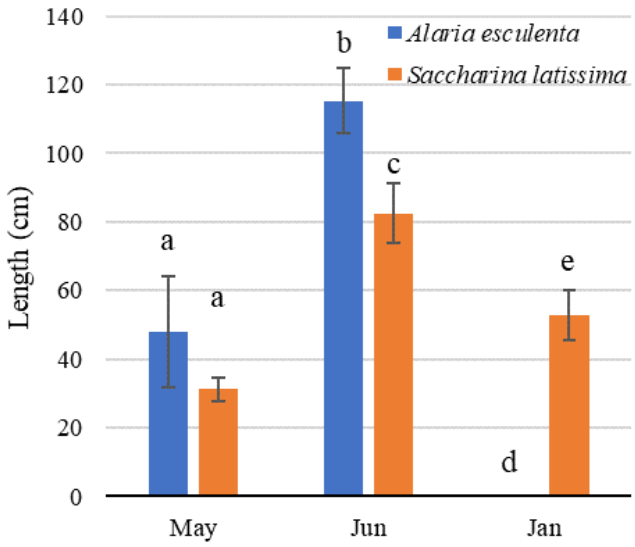


Figure 3.17 Mean lengths variation ± standard error for *Saccharina latissima* and *Alaria esculenta* cultivated at an exposed site in the Faroe Islands. 10-meter-long vertical lines were deployed in November 2014, monitored in May and June 2015 and in January 2016 (n=3). Different letters represent significant difference in length.

Through visual inspections of lines, that were not harvested in one year (**Figure 3.11**), we have observed a continuously increase in length over the winter and continued into the spring when the light increased and the non-harvested individuals grow to a size of 4.1 meter. However, growth measurement of these lines was not included in the research plan but based on what has been experienced *in situ* we can discuss theoretically what can or cannot inhibit growth and which other factors will impact growth rates.

When the biomass increases in length there are some challenges to address: 1) the fact that heavier lines would make the rig sink, as biomass has a high drag both down but also sideways under storms and high-speed water flow, 2) space and light limitations (increased interspecific competition), and 3) degradation of old part of the blade, for example during storm periods.

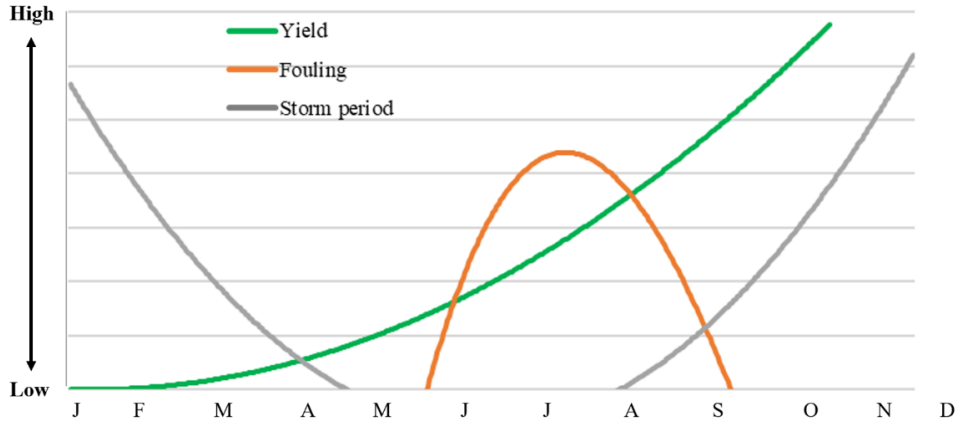


Figure 3.18 A conceptual graph of the relationship between yield, fouling and storm periods over a year. The optimal harvest time will thus depend on the availability to operate at sea, the yield per meter line, and the concentration of fouling (bio fauna and epiphytes).

The challenge of degradation can be partly solved when frequently harvested, as no degradation of biomass observed between harvests as the blade-tip had a straight cut (**Figure 3.13**). Furthermore, the multiple partial harvesting solves the challenge of overloaded rigs, and thus avoid extra cost involved if additional buoys were needed to balance an overloaded rig. The competition for space and light within a population on the lines is lowered when biomass is frequently harvested.

For optimal planning of harvest, these topics must be considered, but also weather conditions and fouling rates (epiphytes and bio fauna) have a high impact on harvesting. The relationship among biomass, weather condition and fouling/grassing is shown in **Figure 3.18**. The optimal harvest time will thus depend on the availability to operate at sea, the yield per meter line, and the degree of fouling/grassing pressure. The optimal harvest months were, therefore, May and June where harvestable biomass, low fouling and calm weather is very likely. July and August are also suitable, where biomass is available and weather is normally calm; though, accepting some fouling or a higher pre-treatment on land to remove shrimps and other undesired organisms. April and September can be used as harvest months at the nearshore sheltered site, on days with suitable weather. April harvests are only possible for the second- or third-year crop. October to March has a high risk of storm periods and calm weather days are mainly used for deployment of newly seeded growth lines, rig deployments and maintenance of the MACR's.

3.3.3 Spacing of macroalgal cultivation rigs

"A small-distance between rigs" experiment was made to understand the effect of 50 m distance among cultivation rigs and therefore two MACR's were deployed at the same time, seeded with same species and method, and treated equally. The difference between rigs was that they were deployed 50 meters apart. A test of homogeneity showed normally distributed data (p=0.94) and a PERMANOVA test showed that they had same relative growth (p=0.16; data not shown). Hence, the small distance between the MACR's, when having the same treatment, will have the same growth pattern.

3.3.4 Optimal cultivation depth

The results showed that *S. latissima* could grow from the surface level (top of the lines) and down to 10 meters below sea level (MBSL) at the nearshore exposed site (**PAPER I)**, having nearly 1 kg dw per meter at 8-9 MBSL when harvested in May 2016 (second growth year), which are quantities not to be ignored.

PAPER I presents the findings of the linear trend line for a yield of *S. latissima* with increased depths when harvested in July/August 2015 (2nd harvest) and May/June 2016 (3rd harvest). The result was that optimal cultivation depth was 19.4 and 18.1 MBSL, respectively. This suggests a cultivation line with a length of 18-19 meters length.

Therefore, an extended growth line was tested *in situ* at the nearshore exposed site using MACR-RUNI having 10 m long growth lines hanging from the fixed line and 20 m down, confirming that growth was possible for both *S. latissima* (**Figure 3.19**) and *A. esculenta* (data not shown) down to 20 MBSL, with an average maximum length for *S. latissima* of 50 cm at 19-20 MBSL (June & August).

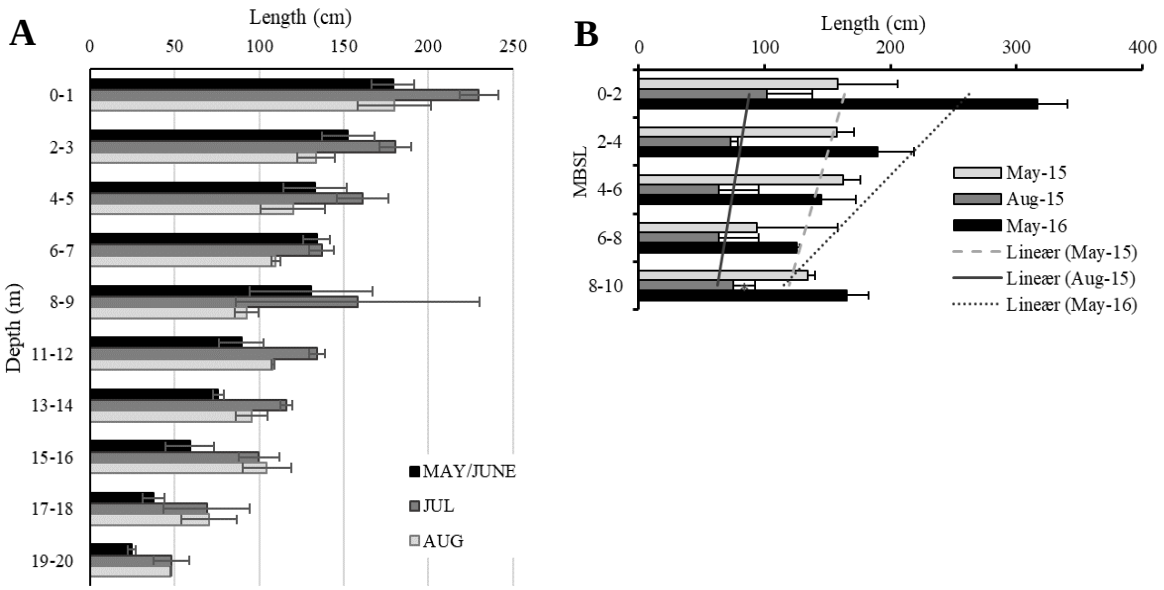


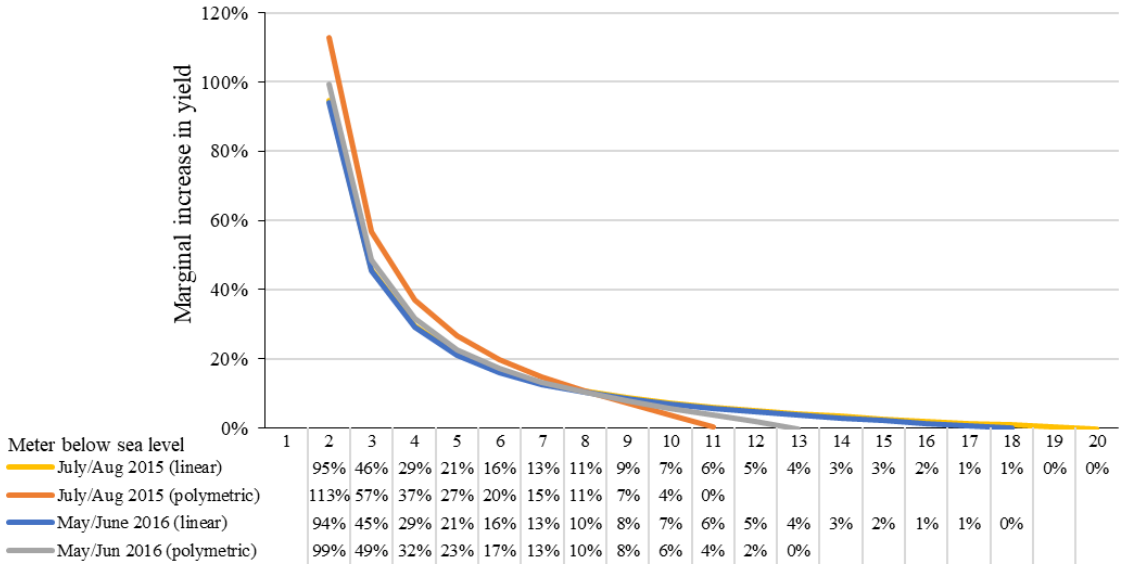
Figure 3.19 Mean length ± SE of cultivated **(a)** *Saccharina latissima* (2017) and **(b)** *Alaria esculenta* (2015-2016) using three replicate lines for monitoring. The biomass was deployed on 10 and 20 m long vertical growth lines offshore in the Faroe Islands. *Saccharina latissima* lines were deployed in Oct 2016 and monitored in May, June and July 2017 (not harvested). *Alaria esculenta* lines were deployed in Nov. 2014 and monitored before harvest in May 2015 and May 2016, and in August 2015 (not harvested after watch). The length was measured in depth intervals of 0–2, 2–4, 4–6, … 19–20m below sea level (MBSL). For *A. esculenta*, linear trend lines were used to show potential growth with decreasing water depths.

The blade morphology of the *S. latissima* at 10-20 MBSL was very different from those growing at 0-10 MBSL. The lower macroalgae had very wide blades and large waves on the sides of the blades, as an adaption to the less turbulent water flow at these depths.

The *in situ* testing of the extended length of the vertical growth lines confirmed that growth is observed down to 20 MBSL, which was also the result of the calculated optimal cultivation depth presented in **PAPER I**.

An calculation of yield as percentages with increased depth-meters below sea level was made (**Figure 3.20**) using the linear trend lines from **PAPER I** (R^2=0.40 and 0.46) and including polymetric trend lines which better described the growth pattern (R^2=0.71 and 0.49). This showed that when the linear trend line was used, increased yield (>2%) is found down to 16 MBSL (linear trend line) but using the polymetric trendline a possibly more realistic result of increased yield (>2%) is found down to 11-12 MBSL.

The extra cost per meter with extended growth lines should consequently be less than the profit gained from the increase in yield for a profitable operation. The cost per meter growth line is discussed in chapter 5 “Seeding of commercially interesting species”.



Meter below sea level	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
July/Aug 2015 (linear)		95%	46%	29%	21%	16%	13%	11%	9%	7%	6%	5%	4%	3%	3%	2%	1%	1%	0%	0%
July/Aug 2015 (polymetric)		113%	57%	37%	27%	20%	15%	11%	7%	4%	0%									
May/June 2016 (linear)		94%	45%	29%	21%	16%	13%	10%	8%	7%	6%	5%	4%	3%	2%	1%	1%	0%		
May/Jun 2016 (polymetric)		99%	49%	32%	23%	17%	13%	10%	8%	6%	4%	2%	0%							

Figure 3.20 The marginal increase in yield (%) with increased cultivation depth below sea level.

3.3.5 Aquaculture output of cultivation structures

The aquaculture output (AO; yield/ha/year), described in **PAPER I** and **II**, is an important tool to compare the capacity and effectivity of macroalgal cultivation structures used at sea. A comparison among the three MACR-versions and theoretical horizontal longlines structures was evaluated (**Figure 3.21** and **Figure 3.22**. The parameters that were used to calculate capacity were: 1) length of growth lines (for horizontal long line structure 1 m was used), 2) spacing between growth lines (for horizontal long line structure 1 m was used), 3) total meter of seeded growth line/ha sea surface, and 4) spacing of rigs including handling space.

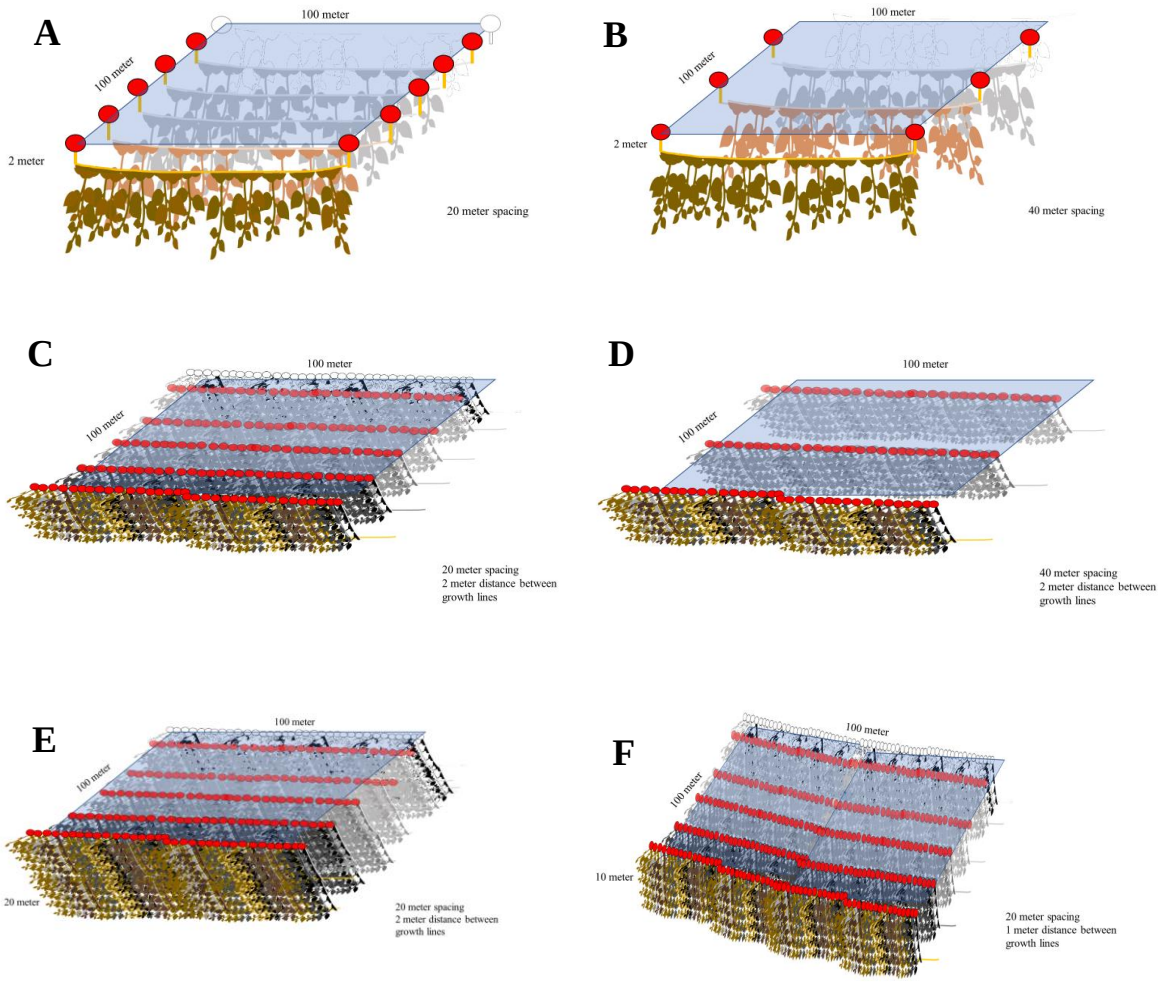


Figure 3.21 (a) Horizontal long-line structure with 20 m structure spacing, and **(b)** with 40 m spacing. **(c)** MACR-RUNI with 20 m spacing between rigs, and **(d)** with 40 m spacing between rigs, **(e)** MACR-RUNI with 20 m vertical growth lines with 2 m spacing of growth lines, **(f)** MACR-RUNI with 10 m growth lines and 1 m spacing of growth lines.

The total capacity of a rig or hectare and the yield as an average yield per meter rope (using data from the entire cultivation line from surface and down to 10 MBSL) was used to calculate the AO. For the horizontal long-line structure the yield was assumed to be the same as the yield found at 0-1 MBSL from the field cultivation in the Faroe Islands. The extended-line scenarios on the MACR-RUNI structures used the calculated yields found from the linear trend-line function "July/Aug 2015" (**Figure 3.21**).

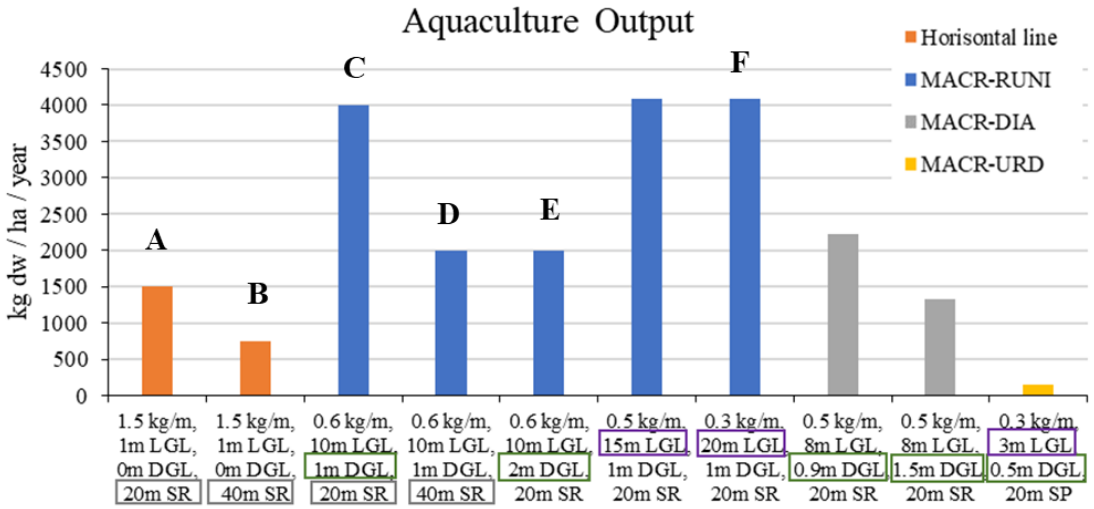


Figure 3.22 Four types of cultivation structures were compared using calculated aquaculture output (kg dw/ha/year). Variables were yield (kg dw/m/year), length of growth line (LGL), the distance between growth lines (DGL), and spacing of rigs (SR). Yield source **Table 3.3** and **PAPER I.** Letters above pillars refers to drawings of the structures in **Figure 3.21**.

The estimation of AO showed that spacing of growth lines and spacing of rig had a major impact. The MACR-RUNI had the highest AO with approximately 4 tonnes dw per year. The scenario, using a longer growth line of 15 or 20 meters, did not change the output significantly. The horizontal rig and the MACR-URD had very low AO compared to the other structures; however, yields described here are much lower than those described for the cultivation in China with a mean AO of 97.4 tonnes ww per hectare per year (~9.7 tonnes dw; Roesijadi et al. 2008). In the light of troubles with MACR-RUNI in nearshore sheltered areas, the MACR-DIA had a considerable AO of 2.25 tonnes dw/ha/year when the spacing of growth lines was 0.9 meters. The AO also indicates the benefits of being in more open-ocean sites. Broch et al. (2019) made an extrapolation of suitable sites in Norway and concluded that offshore sites provided the highest yield.

The results showed that MACR-RUNI is suitable to use in the nearshore exposed sites and MACR-DIA is suitable to use in the nearshore sheltered site; both structures achieved higher productivity than horizontal lines when used in the Faroe Islands, but only half the AO compared to the state-of-the-art in China (Roesijadi et al. 2008).

3.3.6 An unequal yield has a high impact on the operational cost

The harvesting efficiency depends on yield (kg ww) harvested per day/batch and the minutes used for harvesting per day/batch (**Figure 3.23**).

The harvest yield in 2016 was 33 tonnes ww (or 2.3 tonnes dw after sorting and packing the product). The crew of three persons on the boat used a total of 823.2 man-hours or 49,392 minutes in 2016. This was 108 minutes of work per box filled (70 kg). With an hourly cost of € 27 per man, it adds up to € 22,040 in crew wages and a mean cost of € 6.6 of labour cost per kg of harvested macroalgae.

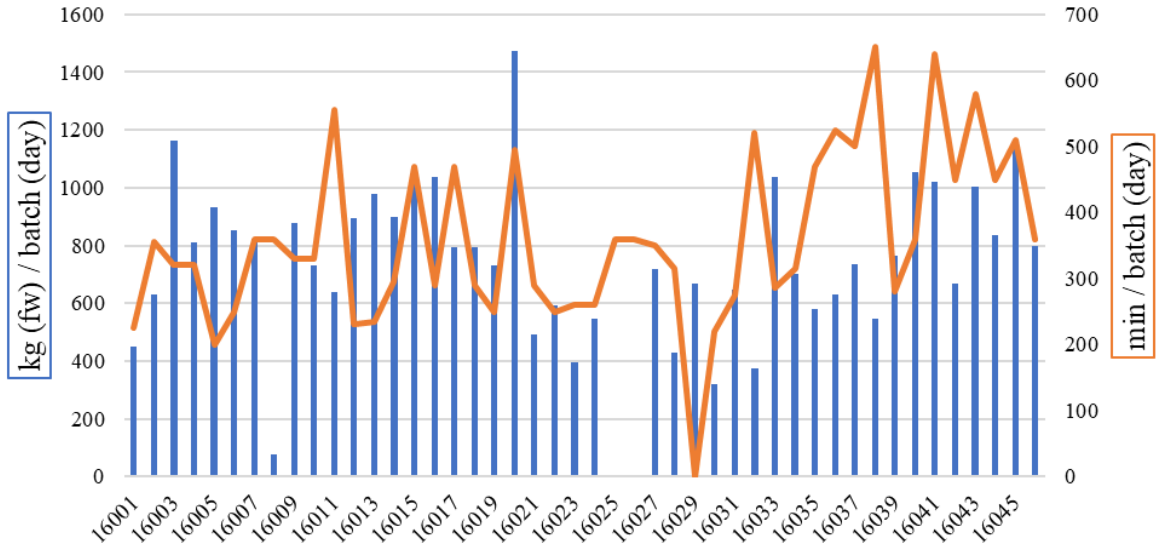


Figure 3.23 Harvest efficiency in 2016.

Batches 16001-16007 were harvested at the sheltered site, which had often calm seawater and was in a short distance from the harbour (sailing time was 30 min). The other batches were harvested from the nearshore exposed site, which often had more unsteady seawater and had a sailing distance from the harbour of 60 minutes each way, which has an impact on the efficiency.

Batches 16035-16045 were harvested in August, where currents played a substantial role in slowing down the work, which did also increase working time. The best result was observed when few lines with high biomass yield could provide a full batch within a short time. A high

and uniform yield on the growth lines is therefore very important for the profitability of a commercial macroalgal cultivation.

3.4 Final remarks

Based on these findings, multiple partial harvesting was for the first time proved to be possible for *S. latissima* and *A. esculenta*. The method is possible when part of the blade is left for regrowth and the macroalgae are cultivated in a site like the Faroe Islands. *Alaria esculenta* was harvested once a year, and *S. latissima* was harvested up to two times a year and in total five harvests were made on MACR 2 at the nearshore exposed site. *Laminaria digitata* started taken over the lines in the second year after the lines were deployed, and yield of *S. latissima* was consequently lower in the fourth harvest compared to the third harvest. From the first to the third harvest the yield increased with time after deployment and eventually the third harvest in spring of the second year after deployment had the highest yield (0.49 kg dw/m; **PAPER I**). The limiting factors of the continuously harvesting year after year are the self-seeding from naturally occurring species and the lifetime of the growth lines.

In 1970's, the Marine Biomass Program in USA investigated of the possibilities of partial harvesting of the giant kelp *Macrocystis pyrifera* (Harger and Neushul 1983), where it was found that if some biomass was left for regrowth then re-grow was possible (discussed in **PAPER II**).

In Spain, the biomass production for annual and biennial cultivation of *S. latissima* was tested (i.e. month 4 and 13 after deployment), making use of the advantages of this species being perennial (Peteiro et al. 2006). Due to lost biomass during winter storms and in summer months because of high mortality due to high summer temperatures, they had lower yield from the bi-annual harvest than from the annual harvest. They did not test repeating harvests of the same line more times in one year or year after year. Their result strengthens our hypothesis of using frequent harvesting, instead of allowing the algae to grow for 1-2 years before harvested as done in Asia.

The stable water temperature and salinity were found as a major reason why perennial cultivation was possible in the Faroe Islands, but also the effect of cultivating in open sea areas with high water flow had an impact.

The results showed that the yield of *S. latissima* and *A. esculenta* were dependent on the cultivation site and maximal cultivation depth. The exposed site had higher yield compared to the sheltered site, which was also proven by Camus et al. (2016), Peteiro and Freire (2013) and Broch et al. (2019). This finding does also correlate with the light result (section 3.1 "Cultivation sites") that had lower intensities at the nearshore sheltered than at the exposed site. The higher light penetration can be explained by a better light transmittance. When the sea is moving and waves are breaking, the otherwise strong surface reflection from calm water, is reduced. Also dissolved organic matter and microalgae that attenuate light are probably higher in the sheltered site because of more shallow water and fewer water masses having a lower diluting effect.

Based on growth results (length and RGR) and yields (provided in **PAPER I)**, we determined that both species had higher productivity in the nearshore exposed site than in the nearshore sheltered site. Although, this result may be strongly affected by the four-months later deployment at the nearshore sheltered site (March) compared to the nearshore exposed site (November). Another parameter with influence on the result was the use of MACR-RUNI in the nearshore sheltered site. MACR-RUNI requires current when operated and at the nearshore exposed site the current direction was along the fix lines and not across, which resulted in tangled lines and lost biomass.

Alaria esculenta had the longest average length (~200 cm) when harvested (**Figure 3.15**); in contrast, *S. latissima* was harvested twice a year and had accumulated the longest total length harvested per year (2 x ~100-160 cm). The yield per meter for *S. latissima* was 0.29 kg dw calculated as mean of 10 m long vertical lines (**PAPER I**). The calculated yield of *A. esculenta* was unrealistic since it was more than five times the yield one could expect (explained in **PAPER I**). The high yield was probably because of self-seeded *A. esculenta* on the *S. latissima* lines that interfere with the yield estimations, and thus states that natural selection and interspecific competition are strong factors and must be included in the operational planning when seeding and harvesting macroalgae.

Natural macroalgal beds are populating coastal sea areas, where they find substrate, from the intertidal zone and down to a depth where light limits their growth. In the Faroe Islands, this is typically at around 10-20 m depth but can be less if water clarity is poor. By using an artificial

substrate placed near the surface it is possible to grow macroalgae in areas that are normally too deep for the algae to grow. The extended lines did however not show a significant increase in AO, and optimal cultivation depth is, therefore, 10 m growth lines, or possibly up to 15 meters lengths. But this was also very dependent on the cost of rope per meter. *S. latissima* cultivated from the surface and down to 20 MBSL had a large variation in morphology as it changes markedly in relation to conditions having wider blades in the bottom and more narrow blades in the top where the wave-exposure was stronger.

The interannual variation was not possible to determine entirely as many variables had an impact for example time of deployment, number of harvests, tangled lines, etc.

A MacroAlgal Cultivation Rig has proved to be suitable for cultivation in nearshore exposed sites and is likely to be useful in offshore sites as well. The AO was low compared to traditionally longline structures used in China. The multiple partial harvesting in up to three years may equalize the cost per tonnes macroalgae, because the Asiatic method requires new seeding every year, whereas the Faroese method can obtain biomass during three years without reseeding. Also, the handling space included for handling a MACR is different to the method used in China where small boats are used and spacing can be closer together. This has a major impact on the AO but efficient use of space becomes less important when the cultivation is moved away from costal areas.

In the comparison of MACR and horizontal lines (**Figure 3.22**), when using growth data from Faroe Islands, the MACR-RUNI had the highest AO. Spacing of growth lines and spacing of rigs was observed to have major effect on the AO. The extended growth line scenario having 15 or 20 m long growth lines instead of 10 meters did not have a significant impact on the AO. The unknown is when the distance between growth lines will have a significant impact on the yield due to the shading effect. This was not monitored in a way that true conclusions can be made. The distance between rigs is limited by the availability to handle the lines, and a distance of 20 m was tested both at the nearshore sheltered and the nearshore exposed site and found suitable, though 40 m are preferred to lower the risk of tangling as long as space and licensing is not an issue.

With the findings of this chapter, new suitable cultivation sites will be in the more offshore or nearshore exposed sites (definition in **PAPER II**). Van der Molen et al. (2018) present a map showing the sea area around the United Kingdom and actually most of the area has a seawater depth below 80 m which is the same depths as observed in Funningsfjørður and therefore we would assume that the MACR would also be suitable in these sites. Broch et al. (2019) present a map showing the sea area around the Norwegian coastline and also here large areas have relatively low water depths, for example 50-200m.

4 The biochemical composition of cultivated macroalgae

The biochemical composition of macroalgae is known to vary between different macroalgal species from strain to strain, during seasonal variations of the hydrologic and hydrochemical conditions (as light, temperature and nutrients), and between geographical areas (Jensen and Jensen 1954, Hagen Rødde et al. 2004, Holdt and Kraan 2011, Stengel and Connan 2015). Most of the existing literature has described the biochemical composition of wild harvested macroalgae, and to some extend their seasonal variation. The composition and seasonal variation for cultivated macroalgae are still not well described and do not exist for site-specific areas like the Faroe Islands. It is therefore not known if cultivated macroalgae that follow an artificial rhythm and are cultivated on ropes have a different composition than wild growing macroalgae, and especially not known for macroalga cultivated in an exposed site like the cultivation site used in the Faroe Islands.

A better understanding and detailed mapping of the biochemical composition inclusive macro- and micronutrients will strengthen the commercial macroalgal cultivation and support the company Ocean Rainforest in when to harvest, make optimal storing, and select commercially interesting species to cultivate, and furthermore decide on product application due to different values for commercial utilisation. Furthermore, such results will add direct value for the company, because it will provide the customers with a better understanding about the purchased products. In a longer perspective, biorefinery processes may be the key for a profitable operation in Europe, and to make this innovation successful detailed mapping of the product composition is also essential.

The purpose of this part of the study was to investigate the biochemical composition of the commercially interesting target compounds found in the brown macroalgal species *Saccharina latissima, Alaria esculenta* and *Laminaria digitata*, and to understand the relationship of seasonal, interannual, cultivation site and depth variations when cultivated in the Faroe Islands. *L. digitata* was included as it was the dominating species that self-seed on the growth lines during the second and third year of cultivation as a result of natural competition.

The results were obtained through a large sampling program and a wide-ranging analyses program (exceeding this PD project) with concluding data analyses in order to use the results as

a tool for an optimised utilization of the biomass. To do so, sampling and labelling methods were developed for optimal storing of samples. The analytical methods applied were always the up-to-date standard methods, but the work did not include optimisation of analytical methods.

The tested variables were: three species, 20 months, two cultivation sites and two cultivation depths (**Figure 4.1**).

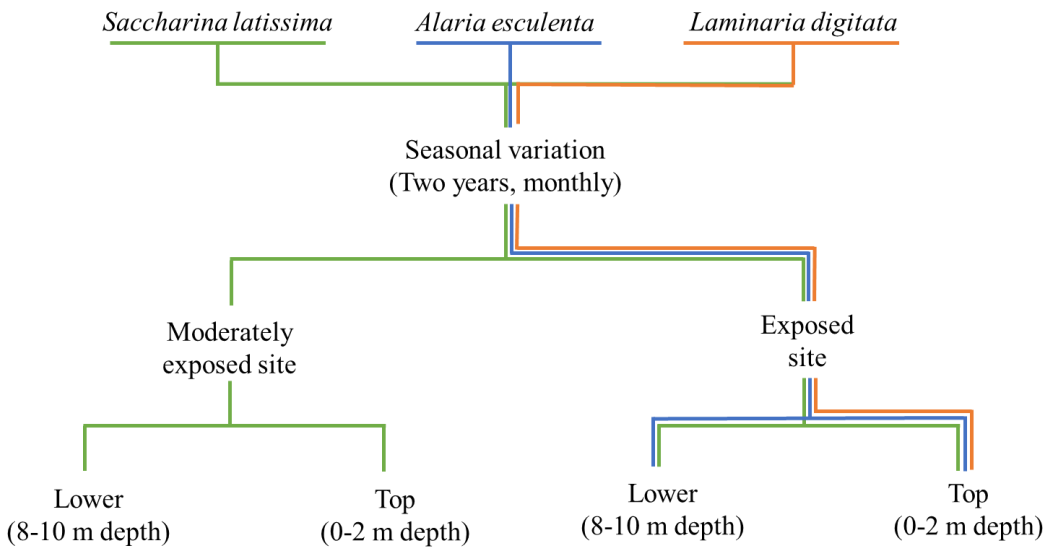


Figure 4.1 The species sampled were three native kelp species. They were sampled monthly over a two-year period at two sites in two different depths. Not all variables were tested for all three species and the figure indicates which parameters that were included for which species.

The biochemical compounds that were included in the analyses were:

- Water (dry matter)
- Minerals (ash)
- Mercury, lead, cadmium and arsenic including inorganic arsenic
- Phosphorus
- Total iodine
- Total proteins
- Nitrogen
- Amino acid composition
- Total lipids
- Fatty acid composition

- Carbohydrates
- Monosaccharides (inositol, xylitol, trehalose, mannitol, fucose, rhamnose, arabinose, galactose, glucose, mannose, sucrose, lactose, xylose and fructose) and calculated alginate (polysaccharide)
- Carbon
- Total polyphenols and the antioxidant activity
- D-vitamin, α- and β-carotene

These target compounds were chosen either because they were present in high concentrations or because they are valuable compounds for possible future utilisation by extraction or in whole biomass.

Water is an essential component in foods in general, and in macroalgae approximately 85% of the biomass of large brown macroalgae species is water (Jensen and Jensen 1954). The water concentration can however vary a lot between species and with seasons (Makkar et al. 2016). When using macroalgae as food, water has high importance as the presence of water will influence the chemical and microbiological deterioration of the harvested macroalgae. Removal of water or freezing of water is essential for the preservation (DeMan 1999), and if the macroalgae are not preserved within hours from harvesting (up to 24 hours), they biomass will start to degrade and lose quality and value. The water concentration was calculated by analysing total dry matter concentration. All other biochemical compounds were thereafter estimated as percentage of the dry matter.

A major fraction of the macroalgae is minerals (ash) that can be found in as high as 50% of dry matter concentration (Schiener et al. 2014). Total mineral concentration was measured as ash by burning/ashing the biomass so only salt and trace elements remained. The major salt components in ash include potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), phosphate (PO_4^{3}), chloride (Cl), sulphate (SO_4^{2-}) and bicarbonate (HCO_3^{-}) (DeMan 1999). These minerals are also major salts of humans and dietary important but were not analysed in this study as they are non-toxic elements. The trace elements that are found in ash can be divided into human's 1) essential nutrients, 2) non-nutritious non-toxic elements and 3) non-nutritious toxic elements (DeMan 1999). In this work, the non-nutritious toxic elements were analysed, but also iodine, as macroalgae are known for their high ability to take up heavy metal and up-concentrate these from the sea. The non-nutritious toxic elements that were analysed were therefore mercury

(Hg), lead (Pb), arsenic (As), and cadmium (Cd). For arsenic, the concentration was specified into inorganic arsenic (iAs) and total arsenic, as the organic arsenic compounds (e.g. arsenosugars, arsenobetaine and arsenolipids) are not or less toxic (EFSA 2009). Inorganic arsenic compound are the sum of arsenite [As(III)] and arsenate [As(V)], which are highly toxic species. Iodine is essential for human, but too high iodine intake can lead to adverse effects (Zimmermann 2009).

Proteins are a main nutrient for human consumption and in animal feed, which are expected to be in short supply in near future, because of the increasing world population. Some macroalgal species have shown to possess significant concentrations of high-quality proteins, and with concentrations similar to conventional protein-rich food (Pangestuti and Kim 2015). Protein concentration is generally higher in red macroalgae (Rhodophyta; up to 47% of dw) and green macroalgal species (Chlorophyta; 10-25% of dw) than for the brown macroalgae (Phaeophyceae; 5-13% of dw; Fleurence 1999). Despite this, the biomass availability in large quantities is more likely for the large brown macroalgae (Laminariales) in future cultivation scenarios in Europe, because this species is suitable for offshore cultivation (as discussed in **PAPER II**). The amino acid composition and the main essential amino acids were also investigated as previous studies have suggested that macroalgae contain similar amino acid qualities as leguminous and egg proteins that are usually considered high protein sources (Fleurence 1999).

Lipids are the fat and oil in food, and the concentration can vary a lot, for example from 0.1% in haddock to 80% in butter. Macroalgae are however not known for high amounts of lipids but they can contain important omega-3-fatty acids as for example eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are essential to humans. EPA and DHA have received a good deal of attention as they can be used in bio-medicaments (DeMan 1999). This work investigated the lipid quantities and fatty acid composition of the cultivated macroalgae for product information to the consumers and to better understand product applications.

Total carbohydrate concentrations of the macroalgae were investigated as carbohydrates represent the major component, together with ash, of the macroalgae dry matter. Schiener et al. (2014) found a total carbohydrate concentration for four brown macroalgal species harvested

from wild populations of approximately 70, 63 and 72% of *L. digitata, S. latissima* and *A. esculenta* biomass, respectively. Parts of the carbohydrates are digestible by humans and therefore provide a source of energy, whereas others are indigestible because they are not degradable by the mammal enzymes and therefore do not provide energy (Miyashita et al. 2013). Indigestible carbohydrates form part of a group of substances known as dietary fibers. Consumption of significant quantities of dietary fibers has been shown to be beneficial to human nutrition, and thus help to reduce the risk of certain types of cancer, coronary heart disease, diabetes and constipation (McClements 2003). As well as being a source of energy and dietary fibers, carbohydrates also contribute to the sweetness, appearance and textural characteristics of many foods (McClements 2003). Furthermore, some macroalgae biosynthesize fucose-containing sulphated polysaccharides, such as fucoidan, which has been reported to possess a variety of biological and health promoting (Miyashita et al. 2013). The carbohydrates of macroalgae have also been described as a good source for bio-fuel (Kraan 2013), bio-plastic (Noreen et al. 2016) and as a fibre source for textile production (Janarthanan and Senthil Kumar 2018). The determination of total carbohydrates and specifically the monosaccharides building blocks was therefore obtained in this work. However, the methods available for analysing the carbohydrate concentration of macroalgae and the poly- and oligosaccharides are still a research objective and to better understand the carbohydrate fraction method development is needed.

Moreover, macroalgae are known for a high vitamin concentration and as having high antioxidant properties that can be used in food, feed and cosmetic products as natural antioxidants and as natural vitamin supplements (Borowitzka and Borowitzka 1989, Holdt and Kraan 2011, Hermund et al. 2015). This work analysed A and D vitamins (vitamin D3, vitamin D2, 25-hydroxy-vitamin D3, 25-hydroxy-vitamin D2, α-carotene and β-carotene) and tested the antioxidant activity by analysing the cultivated macroalgae to better explain the true bioactivity of these specimens and for commercial utilisation.

Finally, carbon (C), nitrogen (N) (not in the ash-fraction) and total phosphorus (P) (included in the ash-fraction) were analysed separately to be able to describe the emission and bioremediation perspectives of macroalgal cultivation.

4.1 Sampling and analytical methods

The macroalgal biomass cultivation took place in the fjord Funningsfjørður as earlier described in section 3.2 “Method and materials”.

4.1.1 Biomass sampling

Monthly sampling was initiated from March 2015 and continued until November 2016 (**Table 4.1**); except in September, November and December 2015 and September and October in 2016, due to periods with storm or technical problems.

The macroalgal biomass samples were harvested using the multiple partial harvest method (section 3.2 **“**Method and materials**”**), and the sample only included the blade, as hold-fast and stipe were not harvested. The biomass sampling methods were applied to simulate the commercial production situation.

Biomass from one meter of growth line was collected for each sample and immediately placed in a plastic bag. Each sample had a weight of approximately 1.5 kg ww and was taken from three identical lines being three biological replicates. If epiphytes were present (always minor) these were not removed.

Alaria esculenta had no biomass during winter and sampling was therefore not possible in those months and it did not grow well in the nearshore sheltered site and samples from this site were therefore few and not analysed. *Laminaria digitata* was first sampled in the second year of growth and only at the nearshore exposed site at the top of the lines (**Table 4.1**).

4.1.2 Sample preparation and information

All samples were brought to the laboratory facilities in Kaldbak and stored airtight and cold overnight (<10 °C). The following day, each sample was grinded separately into pieces of 1-2 cm^2 using a SIRMAN fast chopper C4 VV. The chopper was cleaned carefully with freshwater between each sample. Liquid in the sample bag was included, though always minor or absent. Hereafter, the sample was divided into three sub-samples (A, B and C) and packed air-tight with

a clear label (**Box 1**). The samples were stored at -20 °C until shipment to one of the analyses institutions DTU, Matis or AAU:

- **DTU Food**, Denmark, was responsible for analysing protein/nitrogen, amino acids, vitamins, iodine and inorganic arsenic.
- **Matis**, Iceland, was responsible for analysing total lipids, fatty acids, proteins, carbohydrates, heavy metals, polyphenols and antioxidant activity.
- **Aarhus University (AAU),** was responsible for analysing carbon and phosphorus.

Due to cost limitations, all samples were not analysed for all compounds. The detailed planning of analyses can be seen in section 8.1 "Appendix A: Sampling and analysis plan". The A-samples were shipped to Matis, B-samples were shipped to DTU, and C-samples were either shipped to AUU or stored in Kaldbak as back-up samples.

All samples were freeze-dried (at DTU using the type Martin Christ Gefriertrocknungsanlagen GmbH, Christ E278, type 100800) and homogenized at the respective institutions to a fine-grade powder (<1mm size) using a dry-food grinder (at DTU by a Knifetec 1095 Sample Mill Foss Tecator) for approximately 10-15 seconds after which the homogenates were stored in plastic bags at -20 °C.

4.1.3 Dry matter

To report results as percentage of dry weight (dw), all freeze dried samples were heated in an oven at 103±2 °C for >20 hours. Percentage of dry matter corresponds to start weight minus the weight loss. Reference: ISO 6496-1999.

4.1.4 Ash (minerals)

The samples were ashed at 550 °C for three hours and the residue weighted. Reference: ISO 5984-2002.

Box 1. Labelling ID explanation

Label-ID

Month: the month of sampling

Year: the year of sampling

Random number: A random number between 1 and 40. This number was given each sample when sampled at sea using a water proof note with pre-printed number. The note was placed in the bag.

Subsample letter: After grinding the sub-samples were given a letter A, B or C.

Species abbreviation: *Saccharina latissima* = SL, *Alaria esculenta* = AE, and *Laminaria digitata* = LD.

Examples: MAR15-3A-SL
AUG16-22C-AE

In a spread sheet all samples were registered with the unique Label-ID and information's about cultivation site, cultivation rig, cultivation depth, date of deployment, age, sampling date, analyse category, institute responsible for analyses, status of analyse e.g. "shipped to DTU", and additional comments e.g. tangled line.

Table 4.1 Sampling overview; *Saccharina latissima*, *Alaria esculenta* and *Laminaria digitata* were collected from two cultivation sites in Funningsfjørður ("nearshore exposed site" = E and "nearshore sheltered site" = M) and at two cultivation depths (top and lower). Not all months were sampled, as biomass could be insufficient, or weather conditions made sampling impossible. The numbers in the table represent biological replicates.

	Replicate samples (n)	*S. latissima*	*A. esculenta*	*L. digitata*
Nov-14		Deployment (nearshore **expsoed** site)		
Mar-15		Deployment (nearshore **sheltered** site)		
Mar-15	E Top	2	1	-
Apr-15	E Top	3	3	-
	E Lower	2	2	-
May-15	E Top	3	4	-
	E Mid	-	1	-
	E Lower	3	3	-
	M Top	2	1	-
	M Lower	2	-	-
Jun-15	E Top	3	3	-
	M Top	3	2	-
Jul-15	E Top	3	3	-
	E Mid	3	3	-
	E Lower	3	3	-
	M Top	3	-	-
	M Mid	3	-	-
	M Lower	3	-	-
Aug-15	E Top	3	3	-
	M Top	3	-	-
Sep-15		*no sampling*		
Oct-15	E Top	3	3	-
	E Mid	3	2	-
	E Lower	3	2	-
Nov-15		*no sampling*		
Dec-15		*no sampling*		
Jan-16	E Top	3	-	-
	E Mid	3	-	-
	E Lower	3	-	-
	M Top	3	-	-
	M Mid	2	-	-
	M Lower	1	-	-
Feb-16	E Top	3	2	3
	M Top	3	-	-
Mar-16	E Top	3	3	3
	E Lower	3	3	3
	M Top	3	-	-
	M Lower	3	-	-
Apr-16	E Top	3	3	-
	E Lower	3	1	3
	M Top	3	-	-
May-16	E Top	3	3	3
	M Top	3	-	-
Jun-16	E Top	3	3	3
Jul-16	E Top	3	-	-
	M Top	3	-	-
Aug-16	E Top	3 (2nd year)	3	2
		3 (1st year)	-	-
Sep-16		*no sampling*		
Oct-16		*no sampling*		
Nov-16	E Top	3+3	3	3
Total		**113**	**63**	**23**

4.1.5 Heavy metals: arsenic, cadmium, lead and mercury

The concentration of the heavy metals arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after mineralization of the samples with closed vessel acid digestion. Portions of up to 200 mg was weighed (with 0.1 mg accuracy) of the freeze-dried sample material, together with 3 ml nitric acid (HNO_3) and 1.5 ml hydrogen peroxide (H_2O_2) were transferred to 50 ml digestion bombs. Samples were digested in a Mars5 microwave oven (CEM, North Carolina, USA). The digested sample solutions were quantitatively transferred to 50 ml polypropylene tubes and diluted to 30 ml with Milli-Q water. The concentration of the different elements in these digests was determined by ICP-MS (Agilent 7500ce, Waldbronn, Germany). The indium

nuclide (^{115}In) was used as internal standard. References: The analytical method used is presented in detail in method SV-22-02-SN-1 in Matis Quality manual, also described in (Roleda et al. 2019).

4.1.6 Inorganic arsenic

Determination of inorganic arsenic in the samples followed the standard (EN 16802:2016) issued by European Committee for Standardization (CEN). Briefly, subsamples of macroalgae were extracted with dilute nitric acid and hydrogen-peroxide. The concentration of inorganic arsenic was determined by HPLC-ICPMS using anion-exchange chromatography to separate inorganic arsenic (as arsenate; AsO_4^{3-}) from other arsenic species (e.g. $[AsO_2^-]_n$). References: EN 16802:2016.

4.1.7 Phosphorus

Total phosphorus (P) concentration of the samples was analysed spectrophotometrically according to standard methods (Grasshoff et al. 1983). Prior to analysis, the dried and homogenized samples were heated at 550 °C for 2 h, autoclaved with 2 M hydrogen chloride (HCl) (20 mg DM for 7 ml acid), and finally filtered through GFF filters (Whatman).

4.1.8 Iodine

Samples were analysed for iodine concentration by ICP-MS using alkaline extraction following the standard method EN 15111:2007 issued by CEN. Tetramethylammonium hydroxide (25% w/w $C_4H_{13}NO$) was used for the extraction of total iodine. Single element standard stock solutions of iodine and tellurium were applied in the quantification with 1000 mg/L (Spectrascan, Teknolab AS, Ski, Norway). Blanks (n=2) and *Fucus vesiculosus* material (BCR CD200 Bladderwrack) were also analysed as reference materials. Reference: EN 15111:2007 and further described in **PAPER IV.**

4.1.9 Lipid

Lipid extraction was based on the method developed by Bligh and Dyer (1959). Methylation was based on AOCS Official Method Ce 1b-89 with minor adjustments. Extraction and purification of lipids from biological materials were carried out by mixing macroalgal sample with a chloroform and methanol. Dilution with chloroform and water separates the homogenate into two layers and the chloroform layer contains all lipids and the methanolic layer contain all non-lipids. Reference: AOCS Official Method Ba-3-38, 2009.

4.1.10 Fatty acid composition

A gas chromatographer was used to determine fatty acids in the samples. Fatty acid methyl esters (FAME) were separated on a Varian 3900 GC equipped with a fused silica capillary column (OmegawaxTM250 30m x 0.25mm x 0.25μm film), split injector and flame ionization detector fitted with Galaxie Chromatography Data System, Version 1.9.3.2 software. The oven was programmed as 100 °C for 4 min, then raised to 240 °C at 3 °C/min and was held at this temperature for 15 min. Injector temperature was 225 °C and detector temperature was 285 °C. Helium was used as a carrier gas at the column flow 0.8 mL/min; split ratio, 200:1. Reference: AOAC 996.06.

4.1.11 Nitrogen

The determination of total nitrogen (N) was made by DTU and Matis. The two institutes did not have the same set of samples, but some samples were overlapping and analysed by both institutes (A and B sub-samples). To compare the results between institutions the nitrogen concentration was compared between months during which time both institutes had analysed the N-concentration.

The N-concentrations were normally distributed and had no significant variation between the two institutes (one-factor PERMANOVA, $p = 0.83$). Hence, the N-results were merged. If a sample was analysed by both institutions (i.e. both A and B sub-sample had been analysed), the mean of these two results was used. By using the merged data sets more months, sites and depths were covered and the total nitrogen was therefore better described.

The total nitrogen concentration was determined by a nitrogen combustion method, the Dumas method (Jung et al. 2003), that was described in **PAPER III**.

The crude protein concentration was determined by multiplying total N by the nitrogen-to-protein conversion factor 6.25. This is based on the assumption that samples contain protein with 16% nitrogen and an irrelevant amount of non-protein nitrogen such as nitrate, nitrite, and ammonia (Mariotti et al. 2008).

4.1.12 Protein and amino acid composition

To determine the quality of macroalgal protein of the samples, the amino acid profiles were analysed. Samples were hydrolysed at 110 °C for 1 hour with 6 M HCl in a microwave oven (Microwave 3000 SOLV, Anton Paar). Afterwards a derivatization using a Phenomenex EZ:faast amino acid analysis kit was made according to the user's manual (Phenomenex). The final amino acid composition was determined by liquid chromatography using mass spectrometry (Agilent 1100 Series, LC/MSD Trap) with an EZ:faast 4u AAA-MS column (250 x 3.0 mm, Phenomenex).

Determination of the total macroalgal protein concentration was calculated by summing up the amino acids in moles as recommended by Angell et al. (2016) minus the water mass (18 g

H_2O/mol amino acid) that was integrated during the disruption of the peptide bonds (Diniz et al. 2011). This total protein was hereafter called AA-protein. Further described in **PAPER III.**

4.1.13 Carbohydrates and monosaccharides

Total carbohydrates were calculated as the residual after withdrawing ash, lipids and crude protein (N*6.25) from the total dry matter concentration.

The monosaccharides were determined using the method described by van Wychen and Laurens (2016). This procedure was originally optimized for terrestrial biomass but has been modified by Matis to apply to freeze-dried algal biomass. The dried macroalgal sample was weighted into glass tube using 10 mg and dissolved in 5 mL of Milli-Q water. 100 µL was taken out of the tubes and kept in Eppendorf as control samples. 350 µL of 72% H_2SO_4 was added to the samples and tubes were incubated at 100 °C for 3 hours. After cooling, 1 mL hydrolysate was transferred to 15 mL falcon tubes and neutralized until pH=6 with 0.1M $Ba(OH)_2$. Tubes were centrifuged at 4000 rpm and 5 °C for 5 minutes. The supernatant was filtered through 0.2-0.4 µm filter. Samples were separated on Dionex IC 3000 using CarboPac PA 20 column. Peaks were analysed with Cromeleon 7 software using 14 different monosaccharide standard curves: inositol, xylitol, trehalose, mannitol, fucose, rhamnose, arabinose, galactose, glucose, mannose, sucrose, lactose, xylose and fructose. Reference: van Wychen and Laurens (2016).

The alginate concentration and other unknown polysaccharides (fibres) were calculated as total monosaccharide measured (T monosaccharides IC) subtracting calculated carbohydrates.

4.1.14 Carbon

Concentrations of carbon of the freeze-dried samples were analysed by Pregl-Dumas ignition in pure oxygen atmosphere followed by chromatographic separation of carbon with detection of the individual elements by thermal conductivity (Bruhn et al. 2016).

4.1.15 α- and β-carotene

The analytical procedure for quantification of α- and β-carotene in the homogenized, freeze-dried samples was a combination of two methods formerly described for vitamin A and

carotenoids (Leth et al. 2000, Bechshøft et al. 2011). In short, the sample was saponified, cleaned-up by liquid-liquid extraction before final separation and detection by high-performance liquid chromatography couple with UV-detection at 450 nm. External standard was used for quantification.

4.1.16 D-vitamin

The method used to detect and quantify vitamin D3 and 25-hydroxyvitamin D3 was a LC-MS/MS formerly described by others (Barnkob et al. 2019). In short, 1 g was saponified, clean-up by liquid-liquid extraction, clean-up by solid-phase extraction, and derivatized to achieve a better signal before injection on reverse-phase high-performance liquid chromatography (HPLC) coupled with electrospray ionization tandem mass spectrophotometer using internally labelled standards.

4.1.17 Total polyphenols content

The total polyphenol content (TPC) of the macroalgal samples was determined according to the method by Wang et al. (2010), though the method was adapted to microplates (200 µL, 96 wells, MJ Research, USA) and analysed with a microplate reader (POLARstar OPTIMA, BMG Labtech, Offenburg, Germany). A 10 mg/mL solution was made from 100 mg macroalgal extract dissolved in 10 mL Milli-Q water. Two standard curves with serial dilutions of phloroglucinol and gallic acid solution (20 – 100 µg/ml) were used. Total polyphenol content was determined with some modifications. 20 µl of sample was mixed with 100 µl of 0.2N Folin-Ciocalteu (Cat no. F9252) and allowed to stand at room temperature for 5 min. Then 80 µL of 7.5% Na_2CO_3 was added and absorbance was read at 720 nm with a microplate reader (POLARstar Optima BMG labtech, Offenburg, Germany). Gallic acid (Sigma, cat no. G7384) and phloroglucinol (Sigma, cat no. 79330) were used as standards and results were given as gram of gallic acid equivalent (GAE) per 100 g of extract and phloroglucinol equivalents (PGE) per 100 g extract. References: Wang et al. (2010) and Roleda et al. (2019).

4.1.18 Oxygen radical absorbance capacity

The oxygen radical absorbance capacity (ORAC) assay was performed according to Huang et al. (2002), though the method was adapted to microplates and analysis with a microplate reader (POLARstar OPTIMA, BMG Labtech, Offenburg, Germany). The microplate was incubated for 15 min at 37 °C and after incubation 30 µL of 120 mM AAPH solution was added rapidly using a multi-channel pipette to initiate the oxidation reaction. The fluorescence was recorded every 0.5 min for the first 40 cycles and every min for the last 60 cycles. The filters used for excitation were 485 nm and 520 nm for emission. The total time for the measurement was 80 min. The ORAC value was calculated and expressed as micromoles of Trolox equivalents (TE) per gram of protein or extract using the calibration curve of Trolox. References: Huang et al. (2002) adapted to microplates and analysis with a microplate reader.

4.1.19 Metal chelating

The chelating activity on ferrous ion (Fe2+) was determined according to the method of Boyer et al. (1988) with slight modifications. Samples dissolved in water (100 µL) were mixed with 50 µL of 2 mM ferrous chloride and 100µL of 5 mM ferrozine for 30 min at room temperature. The absorbance was read at 560 nm using a microplate reader (POLARStar OPTIMA, BMG Labtech, Offenburg, Germany). The metal chelating activity was calculated as follows:

$$\text{Chelating activity (\%)} = \left(\frac{Abs_{Blank} - (Abs_{Sample} - Abs_{Control})}{Abs_{Blank}}\right) \times 100$$

where "Abs" is the absorbance of the blank, the sample and the control at 520 nm, respectively. Reference: Boyer et al. (1988).

4.1.20 Reducing power

The macroalgal samples were dissolved in purified water to give a 10 mg/mL solution and centrifuged for 5 min at 4000 rpm. The reducing power was measured according to Oyaizu (1986) adapted to microplate format. A sample (13 µL) was mixed with 63 µl of phosphate buffer (0.2 M, pH 6.6) and 63 µl of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. After 30 min incubation at 50 °C, 63 µl of 10% trichloroacetic acid mixed with 40 µl of ferric chloride solution (0.1%) was added and absorbance measured at 720 nm in a microplate reader

(POLARstar BMG Labtech). The relative reducing power of the sample was calculated after ascorbic acid standards (0–200 µg/ml) as mg of ascorbic acid equivalents per g sample. Reference: Oyaizu (1986) and Apak et al. (2013).

4.1.21 DPPH radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was determined as recommended by Sharma and Bhat (2009). Extract was dissolved in 70% ethanol and centrifuged at 5000 rpm for 5 min, 150 µl of the supernatant was collected and mixed with 50 µl of 0.2 mM 2,2-diphenyl-1-picryhydrazyl (DPPH, Sigma cat. #D9132) in methanol and 70% ethanol solution. A control sample was prepared by using 150 µl of the supernatant mixed with 70% ethanol solution instead of DPPH solution. A blank was prepared by mixing 150 µL of 70% ethanol and 50 µL DPPH and a control blank consisted of pure 70% ethanol. Absorbance was measured for 30 min at 520 nm with a microplate reader (POLARstar Optima BMG labtech, Offenburg, Germany). The scavenging effect is expressed as:

$$Scavenging\ effect = \frac{(Abs_{blank} - Abs_{control\ blank}) - (Abs_{sample} - Abs_{control\ sample})}{(Abs_{blank} - Abs_{control\ blank})} \times 100$$

where "Abs" is the absorbance of the blank, the sample and the control at 517 nm. Reference: Sharma and Bhat (2009) and Pérez and Aguilar (2013).

4.1.22 Statistical analyses

All data was expressed as mean ± standard deviation (SD).

PRIMER+ v7 with PERMANOVA add-on package was used as statistical software. The biochemical concentrations were tested for homogeneity of variance using PERMDISP. If the data was not normally distributed, all data was transformed using square root, fourth root or log(x+1) to achieve homogeneity of variance. Afterwards the data was analysed by permutational analysis of variance (PERMANOVA; Do Monte Carlo Test) using Euclidian distances and unrestricted permutation of data. Whenever a significant difference between sample means or interaction of factors was revealed by PERMANOVA a pair-wise comparison among levels of factors, including within individual levels of other factors in case of significant

interaction, was performed to compare the influence from year, season, site and depth on the compositions. Means were considered significantly different when levels of $p<0.05$ were obtained.

4.2 Results and discussion

In this section the results of the biochemical composition of *S. latissima, A. esculenta* and *L. digitata* are presented and discussed including a statistical analysis of the variation of compounds among seasons, years, cultivation sites and depths.

The section is structured into four parts: 1) an overview of all analysed compounds, 2) the dry matter concentration, 3) the seasonal variation of the four major compounds that add up the dry matter fraction (ash, carbohydrates, protein and lipids), and finally 4) single compounds or groups of compounds are presented and discussed.

For most of the analysed compounds a full scientific paper could be written, like it was done for protein and amino acids in **PAPER III** and for iodine in **PAPER IV**. However, the results were here presented in their short form with most important findings stated.

4.2.1 Overview of analysed compounds: a product documentation

An overview of the biochemical composition of the cultivated macroalgae was made including a statistical analysis of the annual, seasonal, site and depth variation of each compound. Based on these results a recommendation was made to the company on how to use the results as product documentation to customers. The overview for each macroalgal species is presented in tables (**Table 4.2, Table 4.3** and **Table 4.4**).

Overall, cultivation site and depth had no significant influence on the biochemical composition of the macroalgae. Neither had annual variation a significant impact on the biochemical composition. In contrast, many of the compounds had a significant seasonal variation and the table overviews were therefore presented using the seasons: spring, summer, autumn and winter.

The winter season (November-February) had normally no harvesting activities, this season is therefore mostly included as extra information, and results will most likely never be

commercially relevant as biomass was strongly reduced or absent during winter and moreover weather is often too harsh for working at sea in that time of the year. The spring season (March-May) was the period where first harvest of the year occurred. The summer season (June-July) was the period of high light and growth and biomass was still being harvested or biomass could re-grow after first harvested. The autumn season (August-October) was where a second harvest of the year could be applied, and biomass increase was still observed until October.

The concentrations of target compounds recommended as reference levels for the cultivated macroalgae were marked by dark-blue frames in the tables, an include either the overall mean or the spring to autumn seasons dependent on seasonal variations of the target compounds. These values are the best estimation of quantities of target compounds of the harvested biomass. Moreover, highest and lowest concentrations of target compounds were clearly marked in the tables using a yellow colouring for lowest concentrations and blue colouring for highest concentrations.

Table 4.2. The biochemical composition of *Saccharina latissima* cultivated in the Faroe Islands showing the overall mean, standard deviation and number of samples that was analysed. The table includes analysed months from March 2015 to November 2016, at two cultivation sites and two cultivation depths. Next columns show the mean of four seasons related to harvest periods (spring, summer and autumn) and winter season where no harvesting occurs. A statistical analysis of variance among seasons, years, sites, depths and all interactions of these (section 8.2 "Appendix B: Statistical results"). If seasonal variation was significant, lowest concentration was marked with yellow colouring and highest concentration with a blue colouring. No colour was used when no significant seasonal variation was found, and the overall mean should be used for product documentation to customers. The dark-blue boxes indicate recommended product documentation to customers. Red boxes indicate interesting target compound with high concentration even out of harvest season.

Saccharina latissima

		All samples			Mean			
					Spring harvest	Summer harvest	Autumn harvest	Winter
Compound	*Unit*	**Mean**	**SD**	**n**	**Mar-May**	**Jun-Jul**	**Aug-Oct**	**Nov-Feb**
Dry matter	*% of fw*	11.7	2.6	98	**10.7**	**11.5**	**12.4**	**13.2**
Ash	*% of dw*	38.9	6.8	113	**42.3**	**38.3**	**36.1**	**36.2**
Arsenic (As)	*ppm of dw*	**57.7**	19.8	54	57.9	42.3	54.8	71.7
Inorganic arsenic (Ias)	*ppm of dw*	**0.20**	0.07	50	0.19	0.17	0.17	0.25
Cadmium (Cd)	*ppm of dw*	**2.30**	1.10	54	2.26	2.50	2.31	2.37
Mercury (Hg)	*ppm of dw*	**<0.03**	0.02	54	<0.06	<0.06	<0.06	<0.06
Lead (Pb)	*ppm of dw*	**0.20**	0.15	54	0.24	0.13	0.23	0.11
Iodine (I)	*ppm of dw*	3998	1670	65	**2825**	**3043**	**3751**	**6263**
Lipids	*% of dw*	**2.4**	1.3	57	2.8	2.2	2.6	1.9
Σ Fatty acids	*% of dw*	**2.9**	2.0	17	3.7	-	2.6	1.9
Σn3	*% of dw*	**0.7**	0.6	17	1.0	-	0.6	0.5
Σn6	*% of dw*	**0.2**	0.1	17	0.3	-	0.2	0.2
Σn9	*% of dw*	**0.4**	0.4	17	0.6	-	0.3	0.2
Crude protein (N*6.25)	*% of dw*	13.0	3.0	63	**13.8**	**13.5**	**13.4**	**11.1**
AA-protein (ΣAA)	*% of dw*	**5.8**	1.6	35	6.6	5.1	6.0	5.3
Nitrogen	*% of dw*	2.2	0.3	63	**2.2**	**2.1**	**2.2**	**1.9**
Carbohydrates calc.	*% of dw*	45.4	6.5	80	**41.7**	**48.0**	**45.7**	**51.1**
Total carbon (AAU)	*% of dw*	**21.7**	4.1	33	20.2	21.7	24.3	22.6
T monosaccharide (IC)	*% of dw*	14.1	9.8	27	**11.6**	-	**21.4**	**8.4**
Alginate and other unknown	*% of dw*	**31.2**	-	-	30.1	-	24.3	42.7
Mannitol	*% of dw*	**5.5**	4.0	36	4.2	5.8	9.4	2.4
Fucose	*% of dw*	**1.9**	2.0	36	1.4	1.9	2.9	1.4
Galactose	*% of dw*	**0.6**	0.7	32	0.6	0.4	0.8	0.7
Glucose	*% of dw*	4.8	3.9	32	**4.5**	**0.6**	**7.3**	**6.2**
Xylose/mannose	*% of dw*	**1.6**	3.0	18	2.6	-	1.2	0.2
Inositol	*% of dw*	**2.1**	0.5	9	2.5	1.9	-	-
Antioxidant activity								
ORAC	*µmol TE/g extract*	**14.2**	9.2	10	15.7	-	15.2	10.7
TPC	*g PGE & g GAE/100g extract*	**0.8**	0.4	13	1.1	-	0.4	0.4
Metal chelating	*mg dw/mL*	**7.8**	2.4	4	7.8	-	-	-
Reducing power	*mg dw/mL*	**5.9**	1.2	8	5.9	-	-	-
DPPH	*mg dw/mL*	**5.9**	2.5	8	5.9	-	-	-
Vitamins								
α-carotene	*ppm of dw*	n.d.			-	-	-	-
β-carotene	*ppm of dw*	**9.49**	5.27	24	8.4	9.1	11.2	8.2
D-vitamin	*ng/g*	**<5**		2	-	<5	-	<5
Total phosphorus (AAU)	*% of dw*	**0.4**	0.2	33	0.5	0.2	0.4	0.7

Table 4.3. *Alaria esculenta* was sampled at the nearshore exposed cultivation site in the Faroe Islands from March 2015 to November 2016. The target compounds were analysed and presented as total mean, standard deviation (SD), sample replicates (n), and mean of data merged into four seasons: spring (Mar-May), summer (Jun-Jul), autumn (Aug-Oct) and winter (Nov-Feb). A statistical analysis of variance among seasons, years, depths and all interactions of these (section 8.2 "Appendix B: Statistical results"). If seasonal variation was significant, lowest concentration was marked with yellow colouring and highest concentration with a blue colouring. No colour was used when no significant seasonal variation was found, and the overall mean should then be used for product documentation to customers. The dark-blue boxes indicate recommended product documentation to customers.

Alaria esculenta		All samples			Mean			
					Spring harvest	Summer harvest	Autumn harvest	Winter
Compound	*Unit*	**Mean**	**SD**	**n**	**Mar-May**	**Jun-Jul**	**Aug-Oct**	**Nov-Feb**
Dry matter	*% of fw*	15.8	2.4	41	**14.1**	**15.3**	**19.0**	-
Ash	*% of dw*	26.1	5.4	58	**28.7**	**27.2**	**22.5**	**20.7**±7.3
Arsenic (As)	*ppm of dw*	**44.2**	8.4	29	43.0	45.9	43.4	47.9
Inorganic arsenic (Ias)	*ppm of dw*	**0.3**	0.1	16	0.3	0.2	0.3	0.6
Cadmium (Cd)	*ppm of dw*	3.6	1.2	29	**4.1**	**4.4***	**2.2**	**2.7***
Mercury (Hg)	*ppm of dw*	**<0.03**	-	29	<0.03	<0.03	<0.03	<0.03
Lead (Pb)	*ppm of dw*	**0.3**	0.2	29	0.3	0.2	0.2	0.3
Iodine (I)	*ppm of dw*	**234.1**	119.2	30	169.4	235.1	316.5	217.0
Lipids	*% of dw*	3.1	1.0	41	**3.6**	**3.08***	**2.4**	**2.20***
Σ Fatty acids	*% of dw*	**2.8**	0.4	9	3.0	-	2.7	-
Σn3	*% of dw*	**1.1**	0.4	9	1.3	-	1.0	-
Σn6	*% of dw*	**0.2**	0.1	9	0.3	-	0.2	-
Σn9	*% of dw*	**0.3**	0.1	9	0.2	-	0.3	-
Crude protein (N*6.25)	*% of dw*	17.5	3.0	43	**18.8***	**16.1**	**16.5**	**17.2***
AA-protein (ΣAA)	*% of dw*	**8.8**	2.5	14	11.2	9.8	8.8	8.8
Nitrogen	*% of dw*	2.8	0.5	43	**3.0***	**2.6**	**2.6**	**2.8***
Carbohydrates calc.	*% of dw*	53.4	6.3	41	**49.8**	**53.3**	**57.5**	**59.9**
Total monosaccharide (IC)	*% of dw*	**16.2**	12.2	20	11.4	-	21.0	-
Alginate and other unknown	*% of dw*	**37.2**	-	-	38.4	-	36.6	-
Mannitol	*% of dw*	**5.9**	3.9	20	4.5	5.0	7.3	-
Fucose	*% of dw*	**0.9**	0.6	20	0.7	1.3	1.1	-
Galactose	*% of dw*	**0.7**	0.6	17	1.1	0.4	0.6	-
Glucose	*% of dw*	**6.6**	3.9	17	5.4	7.0	7.2	-
Xylose/mannose	*% of dw*	**1.9**	3.0	17	3.2	-	0.4	-
Antioxidant activity								
ORAC	*µmol TE/g extract*	**63.6**	40.2	6	-	-	-	-
TPC	*g PGE & g GAE/100g extract*	**3.8**	2.5	6	-	-	-	-
Metal chelating	*mg dw/mL*	**0.7**	0.1	6	-	-	-	-
Reducing power	*mg dw/mL*	**20.5**	16.2	6	-	-	-	-
DPPH	*mg dw/mL*	**2.6**	1.0	6	-	-	-	-
Vitamins								
α-carotene	*ppm of dw*	-		24	-	-	-	-
β-carotene	*ppm of dw*	**22.0**	11.7	14	26.7	10.5	27.1	17.1
D-vitamin	*ng/g*	**<5**	-	1	-	-	-	-

Table 4.4 The biochemical composition of *Laminaria digitata* self-seeded on cultivation ropes at the nearshore exposed cultivation site in the Faroe Islands and sampled February 2016 to November 2016. The target compounds were analysed and presented as total mean, standard deviation (SD), sample replicates (n), and mean of data merged into four seasons: spring (Mar-May), summer (Jun-Jul), autumn (Aug-Oct) and winter (Nov-Feb). A statistical analysis of seasonal variance is shown in section 8.2 "Appendix B: Statistical results". If seasonal variation was significant, lowest concentration was marked with yellow colouring and highest concentration with a blue colouring. No colour was used when no significant seasonal variation was found, and the overall mean should then be used for product documentation to customers. The dark-blue boxes indicate recommended product documentation to customers.

Laminaria digitata		All samples			Mean			
					Spring harvest	Summer harvest	Autumn harvest	Winter
Compound	*Unit*	**Mean**	**SD**	**n**	**Mar-May**	**Jun-Jul**	**Aug-Oct**	**Nov-Feb**
Dry matter	*% of fw*	16.2	2.8	19	**12.5**	**15.0**	**19.5**	**17.7**
Ash	*% of dw*	32.5	5.5	16	**37.9**	**30.3**	**22.1**	**31.3**
Arsenic (As)	*ppm of dw*	**69.7**	28.2	6	51.0	-	-	88.4
Inorganic arsenic (Ias)	*ppm of dw*	-	-	-	-	-	-	-
Cadmium (Cd)	*ppm of dw*	**1.8**	0.8	6	2.4	-	-	1.1
Mercury (Hg)	*ppm of dw*	**<0.06**	0.0	6	<0.06	-	-	<0.06
Lead (Pb)	*ppm of dw*	**0.2**	0.1	6	0.2	-	-	0.2
Iodine (I)	*ppm of dw*	**5361**	1449	18	4847	5509	6522	5890
Lipids	*% of dw*	**1.5**	0.5	13	1.8	0.9	2.0	1.5
Crude protein (N*6.25)	*% of dw*	**15.6**	4.0	13	15.6	10.0	18.2	16.7
AA-protein (ΣAA)	*% of dw*	-	-	-	-	-	-	-
Nitrogen (DTU & MATIS)	*% of dw*	**2.5**	0.6	13	2.5	1.6	2.9	2.7
Carbohydrates calc.	*% of dw*	**54.4**	9.5	13	45.4	58.8	57.8	46.2
Total monosaccharide (IC)	*% of dw*	**24.4**	6.7	5	21.6	-	-	26.3
Alginate and other unknown	*% of dw*	**30.0**	-	-	23.8	-	-	19.9
Mannitol	*% of dw*	**10.1**	2.5	5	10.5	-	-	9.8
Fucose	*% of dw*	**3.7**	1.1	5	4.3	-	-	3.3
Galactose	*% of dw*	**0.8**	0.8	5	1.1	-	-	0.7
Glucose	*% of dw*	**8.6**	6.1	5	3.4	-	-	12.1
Xylose/mannose	*% of dw*	**1.2**	2.1	5	2.5	-	-	0.4

4.2.2 Dry matter

Water is the foremost compound of macroalgae and was measured indirectly by analysing dry matter concentration as percentage of wet weight (ww).

Saccharina latissima had a mean dry matter concentration of 11.8±2.7% of ww, which was lower than for the two other kelp species. *Laminaria digitata* had a mean dry matter concentration of 16.2±2.8% of ww and *A. esculenta* had a bit higher mean dry matter concentration of 16.4±3.7% of ww (**Figure 4.2**). The dry matter of *S. latissima* samples had a significant seasonal variation but no variation between years, sites or depths. The dry matter concentration was lowest in spring and highest in autumn and winter.

The results of the *A. esculenta* samples were not normally distributed, and transformation of data did not change the distribution of dry matter concentration. Consequently, the variation between each factor (years, months and depths) was compared using a one-way PERMANOVA test; thus, not including potential interactions of factors. A significant variation was observed between years when only months with samples in both years were compared. The mean dry matter concentration in 2015 was 17.6±2.6% of ww and in 2016 it was 15.1±4.3% of ww. The results of the one-way PERMANOVA test also showed a significant seasonal variation of the dry matter concentration for *A. esculenta,* but not between cultivation depths. There were no *A. esculenta* samples analysed from the nearshore sheltered site.

The results of the analysed *L. digitata* samples showed a seasonal variation of dry matter concentration (**Figure 4.2**), with lowest levels during spring and highest in autumn and winter.

For all three species the dry matter was lowest when ash was highest and opposite. Dry matter also seems to follow the carbohydrate concentration that is also highest during autumn and winter.

The dry matter of kelps is previously reported to vary from 8-20% (Black 1950, Jensen and Jensen 1954), similar to levels found in this study. The dry matter concentration of the macroalgal product is crucial when the product is sold per weight and up to 30% moisture is tolerated by buyers. As already mentioned, the control of water has a major impact on the microorganism (bacteria, yeast and fungi) growth of the product. A faster process for making the biomass storage stable is therefore required.

Dried or freeze-dried foods has a great storage stability and a water content of about 5-15%. In dried food the water is as bound water. The enzyme activity, mold growth, yeast growth, bacterial growth, hydrolysis, nonenzymic browning or lipid oxidation has different reaction rates in foods as determined by water activity (DeMan 1999). A small experiment was conducted to evaluate the on the dried macroalgal biomass. The water activity of dried macroalgal samples was 0.40 ± 0.01 (n=3) which is very low (the scale goes from 0 to 1). This means there is no growth of microorganisms. The limiting value of water activity for the growth of any microorganism is about 0.6.

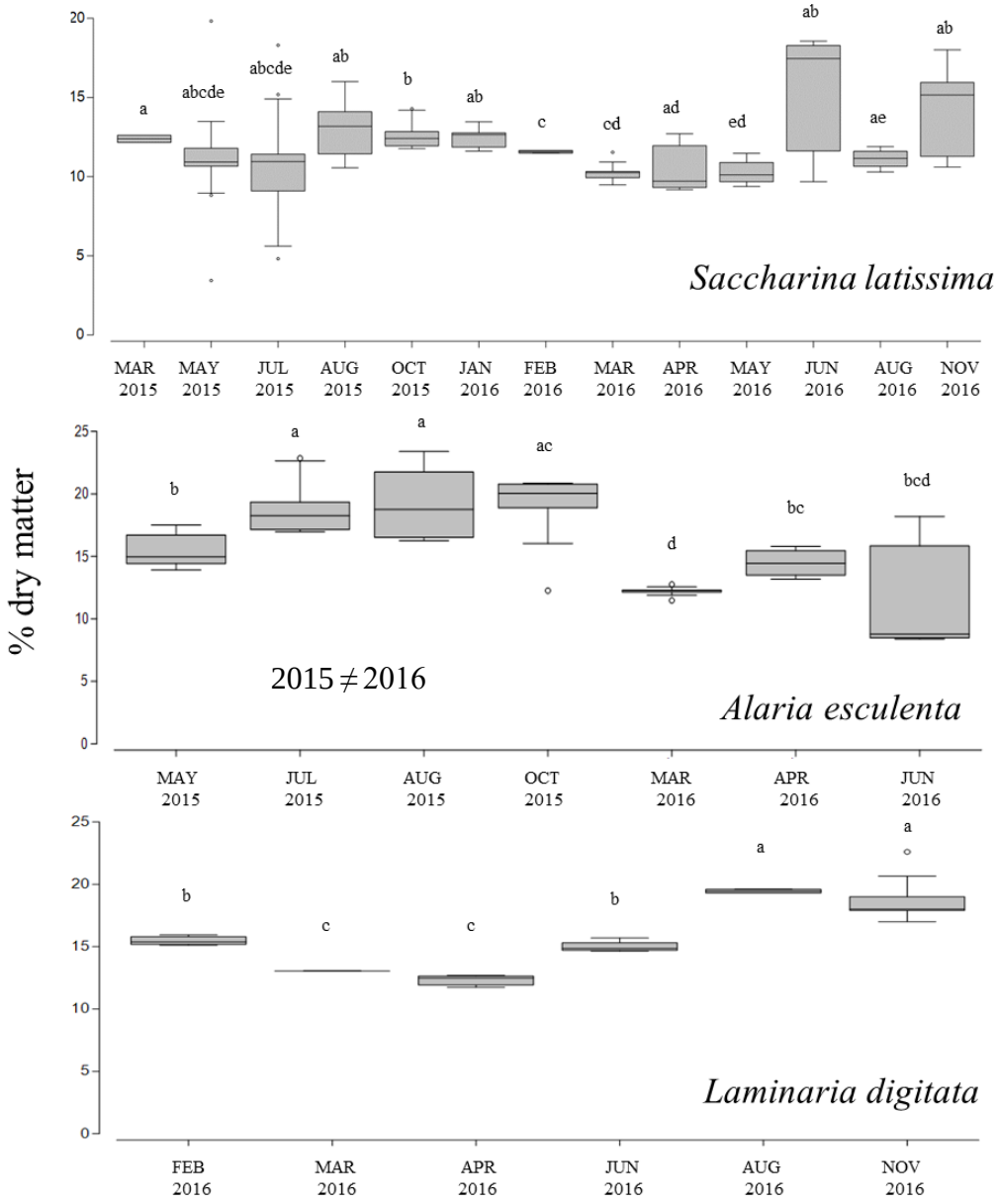


Figure 4.2 The seasonal variation of dry matter in fresh *Saccharina latissima*, *Alaria esculenta* and *Laminaria digitata*. Each of the boxes extends from the first to the third quartile of data. The line in the box is the median, the whiskers are the minimum and maximum values, and the points are out-layers. Different letters above the boxes represent significantly different dry matter concentrations. All samples were collected from the cultivation sites in Funningsfjørður, the Faroe Islands, in 2015-2016. Note that not all months were analysed.

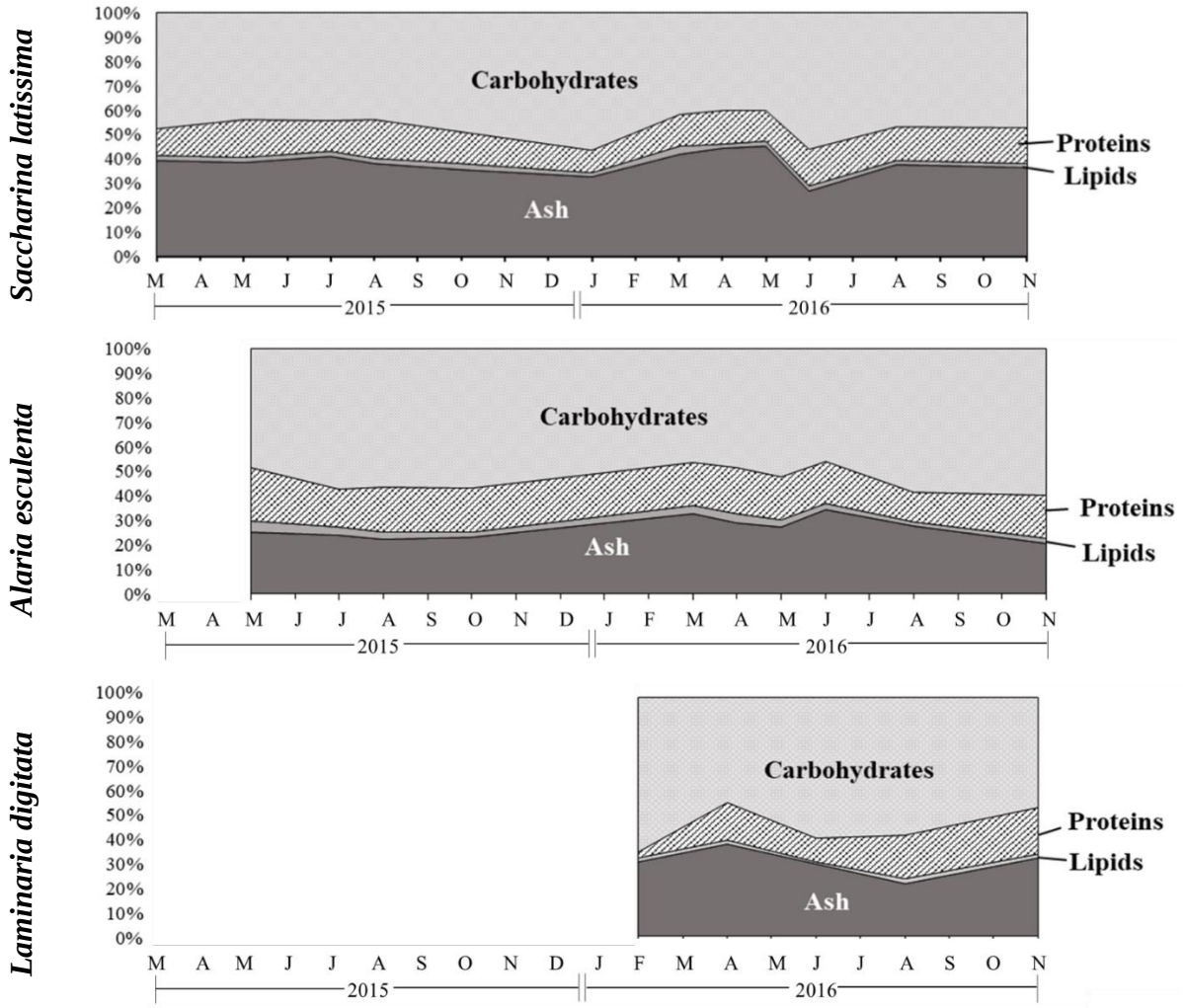


Figure 4.3 A summary of all samples presented for the cultivated macroalgae: *Saccharina latissima, Alaria esculenta,* and *Laminaria digitata*. The concentrations of ash, carbohydrates, protein and lipids are shown as percentage of dry matter. The growth lines used for sampling biomass were deployed in November 2014. The seasonal variation is shown from first sampled until two year after deployment. The number of samples used for each of the graphs differs between species, months and compounds. Details about sample numbers are provided in **Table 4.2**, **Table 4.3** and **Table 4.4**.

4.2.3 Four major components: carbohydrates, protein, lipids and ash

The total concentration of ash, protein and lipid was analysed as percentage of dry matter. Total carbohydrates were calculated as the remaining part of the dry matter concentration. The seasonal variation of these four compounds is shown in **Figure 4.3**. Ash and carbohydrates were the dominating fractions in the range of 20-42% ash of dw and 41-59% carbohydrates of dw. Protein concentration of the investigated kelps ranged from 10.19% of dw and lipids were minor in all seasons and for all species with a concentration of 0.9-3.6% of dw. The trend of

carbohydrates being low when ash and protein is high can also be seen in **Figure 4.3**, and opposite is ash and protein low when carbohydrates are high.

4.2.4 Ash (minerals)

All foods contain varying amounts of minerals (ash) and a significant part of brown macroalgal biomass is the ash concentration, which can account for over 50% of dw (Schiener et al. 2014). The mean ash concentration found in this present study was 38.9±6.9% of dw for *S. latissima*, 26.1±5.4% of dw for *A. esculenta* and 32.5±5.5% of dw for *L. digitata*. For all species there were a seasonal variation in ash concentration and the highest ash concentration was observed in spring and summer and lowest during autumn and winter. This pattern was reverse of the dry matter concentration that generally was highest in autumn and winter and lowest in spring and summer.

The ash concentration for *A. esculenta* was significantly lower in 2015 (23.6±3.5% of dw) than in 2016 (29.4±5.8% of dw), the other species had no interannual variation.

The ash concentrations found in this present study are similar to what was found by others (Jensen and Jensen 1954, Ross et al. 2008, Adams et al. 2011). Ross et al. (2008) found that the ash of Laminariales correlates best with the macro-minerals sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and silicon (Si) and the trace metals iron (Fe), zinc (Zn), manganese (Mn), aluminium (Al), and copper (Cu). The ash concentration of the cultivated kelps is higher than the ash previously found for red and green macroalgae and higher than most terrestrial biomass (Ross et al. 2008). Adams et al. (2011) found the highest ash concentration during winter months – in contrast to the findings of this study.

4.2.5 Heavy metals: arsenic, cadmium, lead and mercury including inorganic arsenic

The non-nutritious toxic elements arsenic, including inorganic arsenic, cadmium, lead and mercury were analysed for the cultivated kelp species to be able to evaluate these macroalgal species as a food and feed source.

The mean concentration of total arsenic in *S. latissima* was 57.7±19.8 ppm of dw, and the inorganic arsenic fraction was as low as 0.20±0.07 ppm of dw. *Alaria esculenta* had a total

arsenic concentration of 44.2±8.4 ppm of dw with an inorganic arsenic fraction of 0.3±0.1 ppm of dw. *Laminaria digitata* had a total arsenic concentration of 69.7±28.2 but with few samples analysed. No seasonal variation was observed for the arsenic concentration for any of the species. Health based guidance values of inorganic arsenic have been established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and are 3.0 μg inorganic arsenic per kg body weight per day (Almela et al. 2002). In order to follow the currently maximum concentration established in France, USA, Australia and New Zealand, the concentration in food products cannot exceed 3 ppm of dw (Almela et al. 2002). Arsenic is most likely taken up by macroalgae as the arsenate and phosphate have a strong chemical similarity and consequently unintentional uptake of arsenate occurs.

Total cadmium had no seasonal variation among seasons for *L. digitata* and *S. latissima* and the mean cadmium concentrations were 1.8±0.8 ppm of dw and 2.3±1.1 ppm of dw, respectively. The cadmium concentration for *A. esculenta* was highest in spring and summer (4.1-4.4 ppm of dw) and significantly lower in autumn and winter (2.2-2.7 ppm of dw). These levels are lower than the recommended maximum level of cadmium in food supplements established by legislation in the EU of 3.0 mg cadmium/kg ww (with 10% dry matter corresponds to ~30 ppm of dw).

The results showed that mercury was not detected in any of the analysed samples. The limit of quantification was <0.03 ppm of dw.

Total mean lead concentration for all three species was 0.20-0.30 ppm of dw. This is lower than the EU maximum level in whole fish (n/a for macroalgae) of 0.30 mg lead/kg ww (with 10% dry matter corresponds to ~3.0 ppm of dw).

The levels of heavy metals found within this work were in similar range as other food sources (Lordan et al. 2011) and none of the measured heavy metals had concentrations above the regulated maximum levels in EU (European Commission 2008, EU 2015). A recent study by Roleda et al. (2019) found similar heavy metal concentrations as this present study for *S. latissima* and *A. esculenta* showing variation between species but seasonal variations were minimal. Opposite to our result that had no variation between two sites in the same fjord, Roleda et al. (2019) found a significant site variation, which is probably related to large distance

between sites (two sites in Norway (Bodo and Trondheim), one site in Iceland and one site in France). The two studies therefore indicate the geographical scale of which separate analyses are required in order to cover site variations of heavy metals.

The results by Roleda et al. (2019) and our study suggest that the health risk from consumption of these macroalgal species poses a low risk for humans with regard to heavy metals. This new data on heavy metals will be a valuable source for future risk assessment of macroalgal application in the food, feed and pharma industries.

4.2.6 Phosphorus

The phosphor concentration of *S. latissima* was 0.4±0.2% of dw and the concentration did not change significantly with seasons, but a significant interannual variation was observed, but only May was compared as this were the only month that was analysed for both years. The phosphorus concentration in 2016 was 0.5±0.2% of dw and in 2015 it was 0.3±0.0% of dw. The differences in phosphorus can be related to natural interannual nutrient differences and/or related to the aquaculture activities in the fjord (i.e. salmon farming) but need further investigation. The phosphorus concentration found for the Faroese *S. latissima* was similar to the concentration found by Marinho et al. (2015c) that was 0.1-0.9 % of dw with highest levels during winter, and higher phosphorus concentrations when epiphytes were included in the analysis.

4.2.7 Iodine

The cultivated kelp species were analysed for total iodine concentration. The results showed a significant variation between species, where *L. digitata* had the highest iodine concentration with a mean±SD of 5,413±1,400 ppm of dw, *S. latissima* had a mean iodine concentration of 3,998±1,670 ppm of dw and *A. esculenta* had significantly lower levels of iodine than the two other species with 248±136 ppm of dw (**PAPER IV**).

Saccharina latissima had a significant seasonal variation in the total iodine concentration with highest iodine levels during winter (January 2016; 7,500±400 ppm of dw) and lowest during summer (June 2016; 2,600±600 ppm of dw). There was no significant variation between the two

cultivation sites or the interaction of season and site and no significant variation between the two cultivation depths.

Alaria esculenta and *L. digitata* had no significant seasonal variation in the total iodine concentration (**PAPER IV**) and for *A. esculenta* no interannual variation (*L. digitata* was only sampled in 2016).

The iodine concentrations correspond well with concentrations previously reported in the literature for wild and cultivated macroalgae ranging from 746-8,165 ppm of dw (Jensen and Jensen 1954, Teas et al. 2004, Schiener et al. 2014, Cheney 2016, Stévant et al. 2018).

The chemical risk assessment of an excessive iodine intake made in **PAER IV** suggests a safe intake level of 0.7-2.1 g dw/day for *S. latissima*, 0.7-1.3 g dw/day for *L. digitata* and 12-35 g dw/day for *A. esculenta.* These intake levels were based on the seasonal variation of iodine, the established tolerable upper level of consumption and a 17% bioavailability of the iodine (Romarís-Hortas et al. 2011). Based on the recommended safe intake of these macroalgae, a moderate consumption will improve the iodine status in iodine-deficient populations (Nitschke and Stengel 2016, Stévant et al. 2018).

4.2.8 Lipids and fatty acid composition

Generally, the lipid content is very low for all macroalgal species: between 0.9% and 4% of dw (Fleurence 2016, Levine and Fleurence 2016).

The lipid concentration of the three macroalgal species was also low (1.5-3% of dw). For *A. esculenta* the concentration had a seasonal variation, but there was no seasonal variation of the other two species. *Saccharina latissima* had no site or depth variation of the lipid concentration. The lipid concentration found in this present study was similar to concentrations found by Marinho et al. (2015d) though their results has a seasonal variation. Marinho et al. (2015d) did also analyse cultivated *S. latissima* and found lipid concentrations from 0.6-0.9% of dw in July to 3.3-3.4% of dw in November.

The composition of fatty acids was analysed for *S. latissima* and *A. esculenta* (**Figure 4.4** and **Figure 4.5**). The largest percentage of total fatty acids for *S. latissima* was 18% palmitic acid

(C16:0) and 13% oleic acid (C18:1n9). Also, the most appreciated health beneficial polyunsaturated fatty acids (PUFA's), eicosapentaenoic (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6n-3), was found with 7% EPA and 1% DHA of total fatty acids. *Alaria esculenta* had 15% stearidonic acid (C18:4n3), 13% palmitic acid (C16:0), and 13% EPA of total fatty acids as the main fatty acid compounds. DHA was below 1% of total fatty acids for *A. esculenta*. There was no significant seasonal variation revealed for the total fatty acids, total n3, n6 and n9 fatty acids. March 2016 (second year of cultivation) had the highest EPA concentration for both *S. latissima* and *A. esculenta* with 46.9 mg/kg dw and 48.5 mg/kg dw, similar levels to some fish species.

Marinho et al. (2015c) found a significantly different fatty acid profile in January compared to all other months analysed. The dissimilarity that they revealed were mainly explained by changes in the relative abundance of EPA (13.12%‑33.35%), stearic acid (C14:0) (11.07%‑29.37%) and oleic acid (18:1n-9) (10.15%‑16.94%). PUFA's made up more than half of the fatty acids in July (52.3%‑54.0%) including EPA and DHA, but also arachidonic acid (ARA) and stearidonic acid (SDA), which are not found in land vegetables such as cabbage and lettuce (Marinho et al. 2015c). Arachidonic acid (ARA; 20:4n3, C20:4n6)) and stearidonic acid (C18:0; SDA) was also found in this present work though in low amounts (1-2% of total fatty acids).

Although the investigated macroalgae have significantly lower lipid contents (1.5-3% of dw) than for example marine fish (20%), they are still a potential source of functional lipids due to a larger biomass potential and as a source of the EPA fatty acid that is essential to humans.

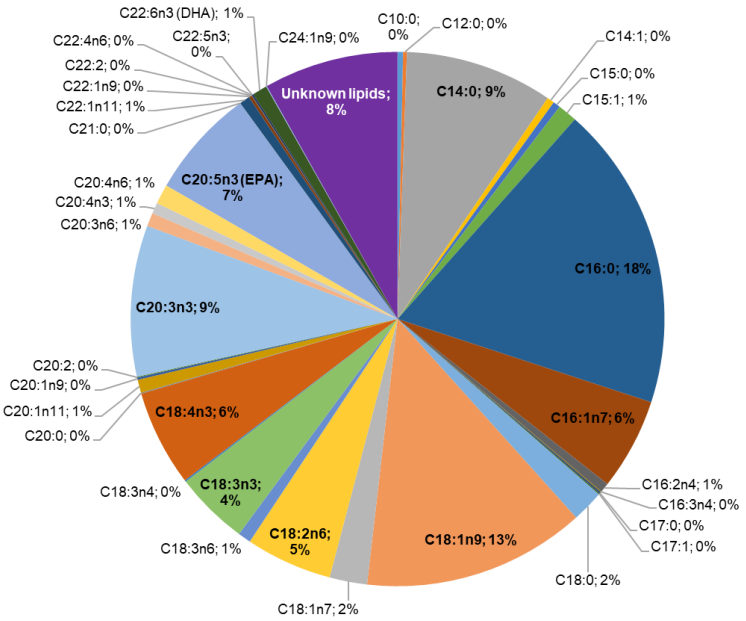


Figure 4.4 The part of the analysed fatty acids of total lipids as mean of all analysed samples from 2015-2016 for the cultivated Faroese *Saccharina latissima*, (% of total lipid content).

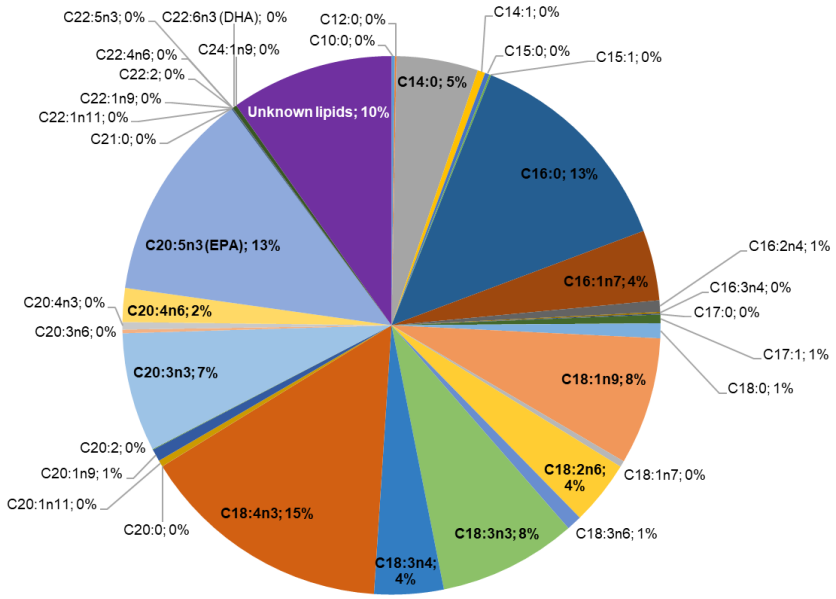


Figure 4.5 The part of the analysed fatty acids of total lipids as mean of all analysed samples from 2015-2016 for the cultivated Faroese *Alaria esculenta*, (% of total lipid content).

Table 4.5. The fatty acid content of *Saccharina latissima* (n=17) and *Alaria esculenta* (n=9) cultivated in the Faroe Islands and the seasonal variation. n3, n6 and n9 fatty acids are highlighted.

Saccharina latissima

Fatty Acids	**2015**			**2016**	
mg/kg dw	**Aug**	**Oct**	**Jan**	**Mar**	**Apr**
C10:0	1.2	0.7	0.4	2.2	0.7
C12:0	0.0	1.2	0.0	2.2	0.1
C14:0	27.3	30.1	10.8	51.4	18.1
C14:1	1.4	1.6	0.5	2.7	0.7
C15:0	1.9	1.8	0.4	1.8	0.8
C15:1	0.4	0.1	17.6	0.0	0.1
C16:0	46.9	59.9	21.0	121.2	35.5
C16:1n7	16.4	22.6	5.5	29.5	11.5
C16:2n4	1.2	3.5	0.9	1.3	1.9
C16:3n4	0.0	0.2	0.1	0.1	0.4
C17:0	0.7	0.4	0.1	0.2	0.1
C17:1	0.2	0.3	0.3	0.3	0.1
C18:0	4.9	4.8	3.3	9.3	7.4
C18:1n9	25.8	35.5	20.9	106.5	18.9
C18:1n7	1.6	5.0	24.1	1.2	3.2
C18:2n6	7.8	15.2	13.1	37.4	7.2
C18:3n6	0.3	1.4	2.3	4.5	3.7
C18:3n3	6.6	10.7	9.5	31.7	10.3
C18:3n4	0.5	0.8	0.0	0.0	0.0
C18:4n3	8.3	17.5	13.1	42.3	8.3
C20:0	0.4	0.6	0.0	0.0	0.0
C20:1n11	0.9	6.0	1.3	3.3	0.7
C20:1n9	0.9	0.7	0.1	0.4	0.0
C20:2	0.4	0.2	0.2	0.0	0.0
C20:3n3	4.6	27.9	21.5	80.5	6.0
C20:3n6	0.6	0.8	1.7	3.1	6.4
C20:4n3	0.9	0.9	1.1	2.7	4.5
C20:4n6	8.8	3.9	0.0	0.0	5.7
C20:5n3 (EPA)	10.8	20.6	15.4	46.9	8.4
C21:0	0.0	0.1	0.0	0.0	0.0
C22:1n11	0.9	7.9	0.2	0.3	0.4
C22:1n9	1.2	0.8	0.1	0.2	0.3
C22:2	0.0	0.2	0.0	0.0	0.0
C22:4n6	0.1	0.0	0.0	0.0	0.0
C22:5n3	0.6	0.5			0.6
C22:6n3 (DHA)	2.1	6.9	0.5	3.7	0.5
C24:1n9	0.2	0.4	0.0	0.0	0.1
Total n3	27.4	74.4	51.6	176.1	28.3
Total n6	17.6	21.3	17.0	45.0	22.9
Total n9	28.1	37.4	21.2	107.1	19.4
Total fatty acids	**187.0**	**291.8**	**185.9**	**586.8**	**162.5**
Unknown lipids	26.3	28.9	8.6	40.9	20.8
Total Lipids	**213.3**	**320.7**	**194.5**	**627.7**	**183.3**

Alaria esculenta

Fatty Acids	**2015**		**2016**
mg/kg dw	**Aug**	**Oct**	**Mar**
C10:0	0.7	0.6	0.6
C12:0	0.0	0.3	0.6
C14:0	14.7	17.9	12.9
C14:1	1.6	1.4	1.3
C15:0	1.0	1.0	0.5
C15:1	0.4	0.2	0.4
C16:0	43.9	45.9	33.5
C16:1n7	9.9	25.2	4.1
C16:2n4	0.7	2.4	3.3
C16:3n4	0.1	0.5	0.3
C17:0	0.3	0.0	0.2
C17:1	0.8	0.7	3.3
C18:0	2.7	3.6	2.0
C18:1n9	29.3	20.9	21.0
C18:1n7	0.8	2.4	0.3
C18:2n6	13.4	8.9	13.6
C18:3n6	3.1	3.1	2.2
C18:3n3	25.6	17.6	33.9
C18:3n4	16.6	0.0	22.1
C18:4n3	41.5	42.7	56.9
C20:0	0.0	0.0	0.0
C20:1n11	1.3	1.4	0.8
C20:1n9	6.7	0.1	0.1
C20:2	0.4	0.0	0.0
C20:3n3	31.7	14.3	20.3
C20:3n6	0.9	0.2	1.0
C20:4n3	1.4	1.5	1.2
C20:4n6	0.0	6.8	12.1
C20:5n3 (EPA)	28.8	40.1	48.5
C21:0	0.0	0.0	0.0
C22:1n11	0.0	0.4	0.1
C22:1n9	0.0	0.0	0.1
C22:2	0.0	0.0	0.0
C22:4n6	0.0	0.0	0.0
C22:5n3	0.1	0.1	0.0
C22:6n3 (DHA)	0.5	1.6	0.3
C24:1n9	0.0	0.0	0.0
Total n3	104.1	100.4	127.2
Total n6	17.4	19.0	28.9
Total n9	36.0	21.0	21.2
Total fatty acids	**279.1**	**261.9**	**297.7**
Unknown lipids	27.6	31.1	32.3
Total lipids	**306.7**	**293.0**	**330.0**

4.2.9 Protein and amino acids composition

The total protein concentration was analysed in two ways: as crude protein calculated as total N*6.25 and as the sum of amino acids (hereafter called AA-protein). The crude protein is widely used to compare food products, while the AA-protein is the best estimation of true concentrations (**PAPER III**). To calculate crude protein the total nitrogen concentration was analysed.

The mean nitrogen concentration was 2.1±0.2% of dw for *S. latissima*, 2.8±0.5% of dw for *A. esculenta* and 2.5±0.6 of dw for *L. digitata. Saccharina latissima* had no significant variation between sites, but a single month with significant variation between cultivation depths (March 2016). Both *S. latissima* and *A. esculenta* had a significant seasonal variation with highest nitrogen concentration in spring for *A. esculenta* (3.0% of dw) and in spring to autumn for *S. latissima* (2.1-2.2% of dw) and lowest in winter (2.0% of dw) for *S. latissima* and from summer to winter for *A. esculenta* (2.6-2.8% of dw) (see full seasonal variation figure for *S. latissima* in **PAPER III**).

Schiener et al. (2014) revealed that carbohydrate concentrations were contrary to the protein concentration. This was also observed in this present study. However, Schiener et al. (2014) found highest protein concentrations in winter and lowest during summer; this was opposite in our case. Schiener et al. (2014) suggested that the relationship was controlled by a rapid growth during summer and nitrogen reserves in months. The differences between their study and this present work were graphical location (Scotland vs Faroe Islands) and origin of samples (natural populations vs cultivated macroalgae).

The mean amino acid concentration (AA-protein) was 4.3±0.9% of dw for *S. latissima*, and comparable to other studies (1.5-12.7% of dw; Marinho et al. 2015). There was no depth, site or seasonal variation in AA-protein concentration for the cultivated *S. latissima*, in contrast to what was found in other studies, where seasonal variation was significant (Marinho et al. 2015; Schiener et al. 2015; Wegeberg et al. 2013). The lack of seasonal variation was most likely a consequence of the year-round stable physical conditions in the Faroe Islands. The mean AA-protein concentration was 8.8±2.5% of dw for *A. esculenta*, and not analysed for *L. digitata.*

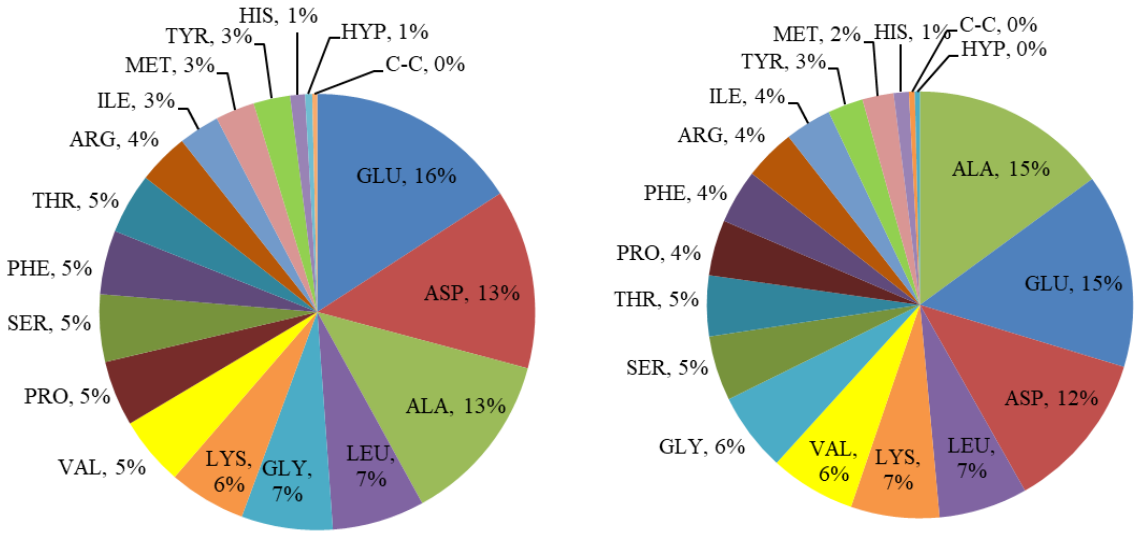


Figure 4.6 All analysed data amino acid data presented as the mean concentration of specific amino acid as percentage of the total amino acids analysed for cultivated *Saccharina latissima* (left, n=35) and *Alaria esculenta* (right, n=15), the Faroe Islands.

The results showed that glutamine is found in highest quantities for *S. latissima* and for *A. esculenta* alanine is found as the amino acid with highest quantities (**Figure 4.6**). The essential amino acids (EAA) were found in quantities of 6-7% lysine, 7% leucine, 2-3% methionine, 4-5% phenylalanine, 5% threonine, 5-6% valine, 1% histidine and 3-4% isoleucine of the total AA-protein. Tryptophan was not detected since the compound was destroyed during hydrolysis. Histidine was in most months the limiting EAA when calculating EAA-score (**PAPER III**). The quality of the protein was high (EAA score >100%) in March, and therefore *S. latissima* is appropriate as a high-quality food and feed product with regard to protein, when harvested in March. Though the total protein concentration is low based on AA-protein the same protein concentration can be harvested all months of the year due to the lack of seasonal variation. Based on the crude protein (N*6.25) the harvest season from March to October has highest protein concentrations for *S. latissima*, and for *A. esculenta* the spring harvest contained highest protein.

4.2.10 Carbohydrates and monosaccharides

Carbohydrates are one of the most important components in foods and also the main food component of the investigated macroalgae.

Total carbohydrates were calculated as the dry matter subtract lipids, protein and ash. *Saccharina latissima* had a mean carbohydrate concentration of 45.4±6.5% of dw, which was lower compared to the two other kelp species. *Laminaria digitata* had a mean carbohydrate concentration of 54.4±9.5% of dw and *A. esculenta* had a mean carbohydrate concentration of 53.4±6.3% of dw.

Five types of monosaccharides were analysed in enough amounts to be detected: fucose, mannitol, galactose, glucose, inositol along with tiny amounts of xylose and mannose. Alginate was not directly monitored in this work due to lack of standards and suitable column, but was estimated as the residue when other monosaccharides had been analysed (for *S. latissima* see **Figure 4.7**).

The monosaccharide analysis was made in two batches. The first batch included samples from March to July 2015 and the second batch included samples from August 2015 to March 2016. This may have influenced the results, for example inositol was only detected from the first batch and xylose/mannose was only detected in the second batch. A third batch was never analysed for monosaccharide, because the carbohydrate analyses showed some problems that was not solved by ordering new equipment.

Carbohydrates are expected to build up during summer where light and photosynthesis are high. Nevertheless, the found results had no seasonal variation except of glucose and total monosaccharides analysed for *S. latissima.* The estimated results of unknown carbohydrates indicate that the unknown constitutes 50-70% of the total carbohydrates and was found in lowest concentrations for *L. digitata,* followed by *S. latissima,* and highest for *A. esculenta* with 37.2% of dw. The mannitol, fucose and glucose concentrations were highest for *L. digitata* with 10.1%, 3.9% and 8.6% of dw, respectively. Regarding *S. latissima,* the glucose was highest from autumn to spring (4.5-7.3% of dw) and lowest in summer (0.6% of dw) Galactose, xylose, mannose and inositol was found in small amounts for all three species.

These concentrations of carbohydrates and monosaccharides were similar to concentrations found in other studies (30-71% of dw; Holdt and Kraan 2011, Wegeberg et al. 2013, Schiener et al. 2014, Marinho et al. 2015) of which most are in the form of dietary fibers. Manns et al.

(2014) expand on suitable analytical methods for carbohydrate determination, and they specify the carbohydrate compounds of Danish kelp collected from wild populations.

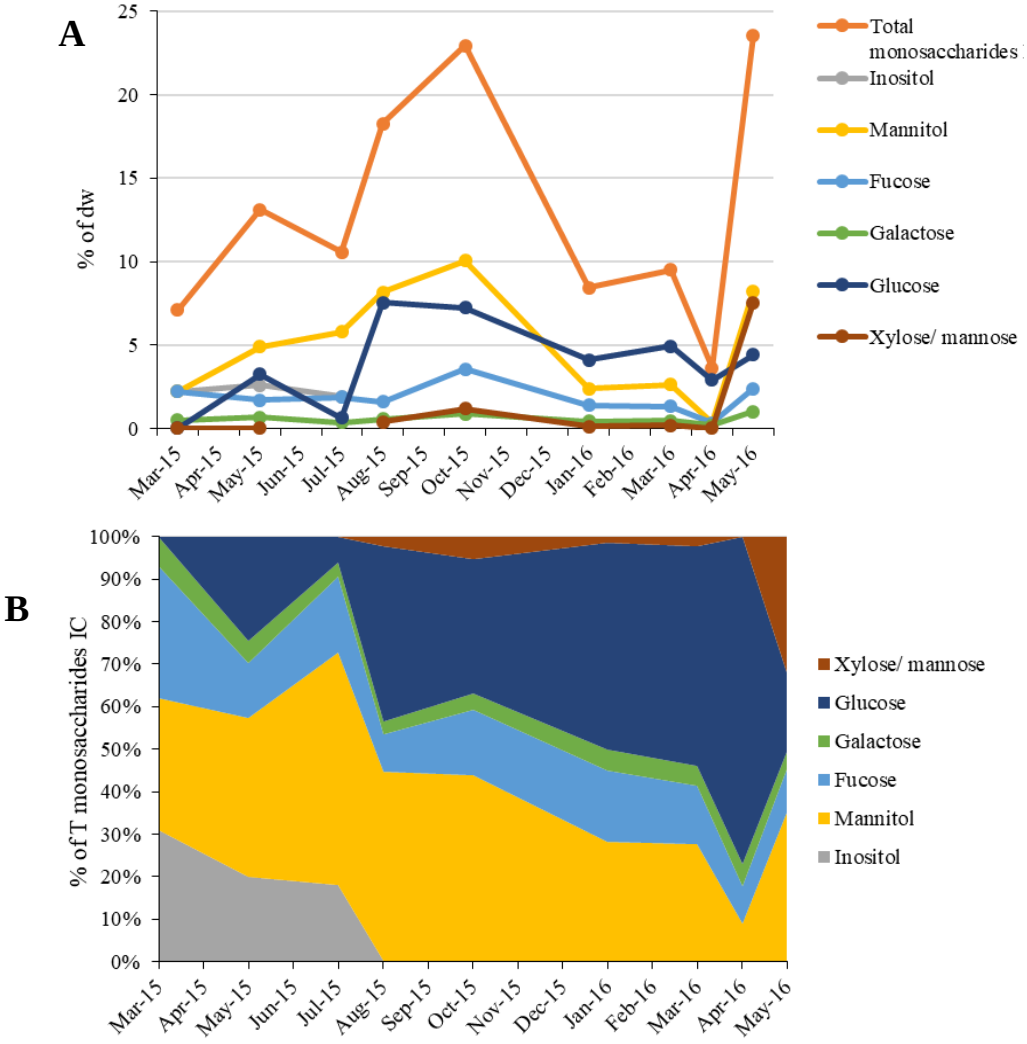


Figure 4.7 Total analysed monosaccharides (IC method) and concentration of each compound for the cultivated *S. latissima* from March 2015 to May 2016 in the Faroe Islands: **(a)** as percentages of the total dry weight (dw) and **(b)** as percentage of total monosaccharides analysed.

4.2.11 Carbon determination

The carbon concentration was only analysed for *S. latissima* and revealed a concentration of 21.7±4.1% of dw. A statistical analysis using PERMANOVA showed no significant seasonal

variation between analysed months. These results will be used later in the evaluation of carbon sequestration.

4.2.12 Vitamins

Foods with a high concentration of vitamins and bioactive compounds are today popular for disease prevention, for example to prevent cancer (Jaswir 2011). Macroalgae have been pointed out as a food with these qualities. However, few data on macroalgal vitamin composition exists. α- and β-carotene can act as bioactive compounds and they are vitamin A precursors. These vitamins were analysed in this study for *S. latissima* and *A. esculenta*.

The amount of α-carotene was too small to quantify, but β-carotene was found in both *S. latissima* and *A. esculenta*. The β-carotene quantified in the samples was all *trans*-β-carotene and not the *cis*-isomer. The *trans*-isomer β-carotene is the most important precursor for vitamin A.

Alaria esculenta had significantly higher concentration of β-carotene than *S. latissima*, and none of the species had a significant seasonal variation ($p > 0.05$). Both species had highest concentration in August 2015 (*A. esculenta*, 31.4±9.8 mg/kg dw; *S. latissima*,12.2±4.6 mg/kg dw), and for *A. esculenta* also April 2016 was high. The lowest concentration was found for *S. latissima* in April 2016 (8.4±2.3 mg/kg dw; **Figure 4.8**).

The qualitative and quantitative carotenoid composition was earlier reported by Haugan and Liaaen-Jensen (1994) for the brown algae *Fucus serratus*, *Fucus vesiculosus*, *Ascophyllum nodosum*, *Pelvetia canaliculata*, *L. digitata* and *S. latissima*. Fucoxanthin was the major carotenoid (primary xanthophyll) and β-carotene the only carotene that was present in all six species. Seasonal variation in carotenoids was found for the brown algal species with a maximum carotenoid concentration in the spring and minimum in the autumn (Haugan & Liaaen-Jensen 1994). To evaluate this trend further analyses are needed for the Faroese cultivated species.

The function of β-carotene was earlier described as a protective “sunscreen” to the chlorophyll and cell DNA from the high light intensities (Borowitzka and Borowitzka 1989). De Sousa et al. (2008) reported α- and β-carotene for five brown macroalgal species with α-carotene

concentrations of 0.3 ppm of ww and β-carotene concentrations of 12.2 ppm of ww. Thus, higher than found in this present study where results are presented per dry weight.

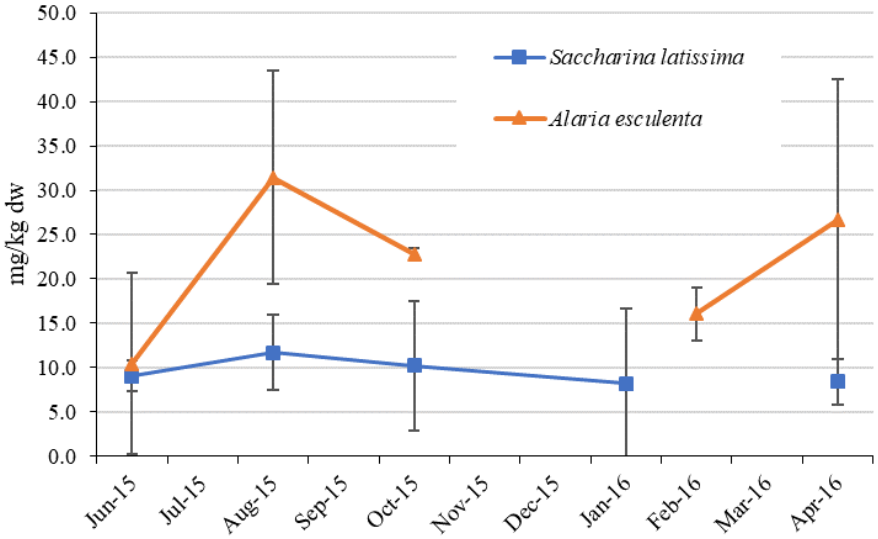


Figure 4.8 Mean β-carotene concentration ± standard deviation for cultivated Faroese *Saccharina latissima* (n=3-6, total number of samples 24) and *Alaria esculenta* (n=2-3, total number of samples 14) and their seasonal variation.

The concentration of D-vitamin was briefly analysed in four samples and very low levels of D-vitamin were found (**Table 4.6**). The difference of the presented "less than" values is due to the sensibility of the respective analyses (D3/D2 and 25-hydroxy compounds) and because D2-vitamin has interference peaks. To identify the peaks further, more analyses are needed together with further purification. Although the low levels of D-vitamin indicate that this is not immediately interesting to investigate.

Table 4.6. Vitamin D in three macroalgal species harvested from open-ocean cultivation in the Faroe Islands.

Species	***Date***	***D3-vitamin***	***D2-vitamin***	***25-hydroxy-vitamin D3***	***25-hydroxy-vitamin D2***
Saccharina latissima	Jun-15	<1ng/g	<5ng/g	<5ng/g	<5ng/g
Saccharina latissima	Jan-16	<1ng/g	<5ng/g	<5ng/g	<5ng/g
Alaria esculenta	Feb-16	<1ng/g	<5ng/g	<5ng/g	<5ng/g
Palmaria palmata	Feb-16	<1ng/g	<5ng/g	<5ng/g	<5ng/g

4.2.13 Antioxidant activity

Research on the bioactives from macroalgae has increased in recent years. Antioxidant activity is one of the most studied, due to the interest of these compounds both as preservatives and protectors against oxidation in food and cosmetics and also due to their health implications, mainly in relation to their potential as functional ingredients (Balboa et al. 2013). Brown algae has been found to have higher antioxidant potential in comparison with red and green macroalgal species and to contain compounds that are not found among terrestrial sources (Balboa et al. 2013).

In this present study, five methods were used to describe the antioxidant activity of *S. latissima, A. esculenta* and *L. digitata*: ORAC, TPC, metal chelating, reducing power and DPPH. No matter the method used, all samples had low antioxidant activity (**Table 4.2** and **Table 4.3**).

Alaria esculenta had a mean TPC concentration of 3.8±2.5 g/100 g extract similar to what was revealed by Zubia et al. (2009) that found a mean TPC of 2.0 g/100 g extract for *A. esculenta*. Roleda et al. (2019) found a mean TPC of nearly 40 g/100 g extract for *A. esculenta.* The mean TPC of *S. latissima* revealed in this present study was significantly lower at 0.8±0.4 g/100 g extract, and not like the concentrations found by Rajauria et al. (2010) that found a mean TPC of 7.6 g/100 g extract for *S. latissima*. This concentration is in agreement with the results found by Roleda et al. (2019) that found Other macroalgal species have a significantly higher TPC concentration. For example, the order Fucales has shown much higher TPC concentrations, for example 40.2 g/100 g extract was reported for *Cystoseira crinita* and 1.5-27.7 g/100 g extract was reported for *Fucus* sp. (Balboa et al. 2013).

4.3 Final remarks

For the first-time, biomass samples of commercial cultivated *S. latissima, A. esculenta* and *L. digitata* were analysed for its total biochemical composition with sampling over a period of 20 months. The results add important knowledge about the biochemical composition of the macroalgae when cultivated in the Faroe Islands; where only few studies have earlier been published. The results are in line with what has been reported by others and a comparison between this study and previously presented results (**Table 4.7**).

The results confirmed a significant variation between compounds of the three macroalgal species, for example iodine was significantly lower for *A. esculenta* than for the two others. Opposite many similarities were found between species, like for example, the dry matter and carbon concentration went up in winter when ash and protein went down, and the opposite during spring and summer.

Seasonal variation was expected among the analysed biochemical compounds for the cultivated macroalgal species *S. latissima*, *A. esculenta* and *L. digitata*, but the findings showed that only some compounds varied among the year. For *S. latissima* seasonal variation of concentration was shown for the dry matter concentration, ash, crude protein, total carbohydrates, iodine, nitrogen, total monosaccharides and glucose. For *A. esculenta* seasonal variation of concentration was shown for the dry matter concentration, ash, cadmium, lipids, crude protein (N*6.25), nitrogen and total carbohydrates. For *L. digitata* seasonal variation of concentration was shown for the dry matter and ash only, though this is probably due to the substantially lower number of samples analysed and less compounds investigated for this species compared to *S. latissima* and *A. esculenta*. For the compounds having a significant seasonal variation, the season-specific concentration marked in the overview tables by blue boxes, was recommended to use when defining the quantities of a compound of the harvested products. For all other compounds with no seasonal variation, the overall mean was recommended to use when defining value of quantities.

Saccharina latissima was sampled at two sites and at two cultivation depths, *A. esculenta* was sampled at one site at two depths and *L. digitata* was sampled at one site at one depth in only one year (i.e. 2016). The cultivation conditions were expected to influence the biochemical compounds as different cultivation depths and sites would provide different nutritious profiles. This was suggested as macroalgae has a strong adaptation to for example light intensities and as they can adjust pigments and accessory pigments. Surprisingly, no significant variation was revealed among the 10 m differences in water depth (and significant different light conditions) for any of the biochemical compounds analysed. Except of total carbohydrates and nitrogen for *S. latissima.* Furthermore, no significant variation was found between site, except of carbohydrates for *S. latissima*.

More samples and analyses are needed to better know these relationships of cultivation conditions for *A. esculenta* and for *L. digitata*. Also, some of the compounds analysed had too few samples analysed to tell the relation of the analysed compound and its variation with site and depths, for example the vitamins.

Table 4.7. Chemical composition of the investigated species *Saccharina latissima*, *Alaria esculenta* and *Laminaria digitata* compared to results found by others: Schiener et al. (2014), macroalgae.ie (2015), Algae A/S product fact sheet (2015). Alginate was calculated as total carbohydrates subtracted the monosaccharides, glucose results was used to express laminarin, and fucose results were used to express fucoidan. Protein was "crude protein (N*6.25)". n.d. = not detected.

	Saccharina latissima		***Alaria esculenta***		***Laminaria digitata***	
Compounds (% of dry weight)	Other studies	Ocean Rainforest	Other studies	Ocean Rainforest	Other studies	Ocean Rainforest
Water (% of wet weight)	82 - 85	82 - 89	70 - 85	81 - 85	78 - 88	80 - 87
Ash	15 - 30	36 - 42	15 - 25	23 - 29	15 - 35	22 - 38
Alginate	25 - 32	31	34 - 41	37	31 - 37	30
Mannitol	14 - 22	6	9 - 15	6	13 - 25	10
Laminarin	3 - 13	1 - 7	5 - 18	7	1 - 13	9
Fucoidan	2 - 12	2	0	1	8 - 12	4
Polyphenols	1	0.8	1.5	3.8	0.2	n.a.
Carbon	21 - 31	22	28 - 31	n.a.	24 - 36	n.a.
Nitrogen	1 - 3	2	2	2.6 - 3	1 - 3	2.5
Protein	5 - 10	11 - 14	9 - 12	16 -19	5 - 8	16
Iodine	2.8 - 4	2.8 - 6.2	0.4 - 1.2	0.2	4.7 - 9	5.4

For now, commercially product documentations are recommended to use the mean concentration of target compound without distinguishing the two sites and depth of cultivation but including seasonal variation when determined.

5 Seeding of commercially interesting species

As mentioned earlier the harvested part of the kelps are the sporophytes, and they are cultivated on for example ropes. Also, *Palmaria palmata* is harvested as a sporophyte but also as an adult male gametophyte as they look very identical. The macroalgae can grow on the ropes since the root looking structures are only organs to provide holdfast, and nutrients are taken up from the entire organism. Seeding of ropes can be done in many ways and are applied for different macroalgal species, as they have widely differenced life cycles (e.g. sporic meiosis, gametic meiosis and zygotic meiosis). Some species are seeded by sexual reproduction and some are utilized by vegetative growth (dividing the tissue).

Commercially, seeding protocols have been developed for kelp species like *S. latissima* and *A. esculenta*, but there has been and still is a need to improve the ability to seed target species like *Palmaria palmata*.

This chapter will address how seeding was optimised for *S. latissima* and *A. esculenta* (first part), and the development of new seeding techniques for *Palmaria palmata* (second part).

5.1 Seeding of kelps – *Saccharina latissima*

Optimised seeding of the kelps *S. latissima* and *Alaria esculenta* was investigated and yield and/or length of the individuals was used to evaluate optimal substrate and seeding method. The work that was made on spore release and cultivation of gametophytes was not included because of non-disclosure agreement (NDA) of the company Hortimare.

Saccharina latissima and *A. esculenta* have a well-known heteromorphic alternation of generations where the diploid sporophyte grows into a large "plant" that is harvestable (**Figure 5.1**). When the sporophyte becomes mature it releases meiospores (female and male from same sporophyte), which settle and develop into microscopic single-sex haploid gametophytes. The male gametophyte produces sperm with flagella and the female gametophytes produce eggs (oogonium). Following fertilisation, an embryonic sporophyte develops, which grow into the adult size.

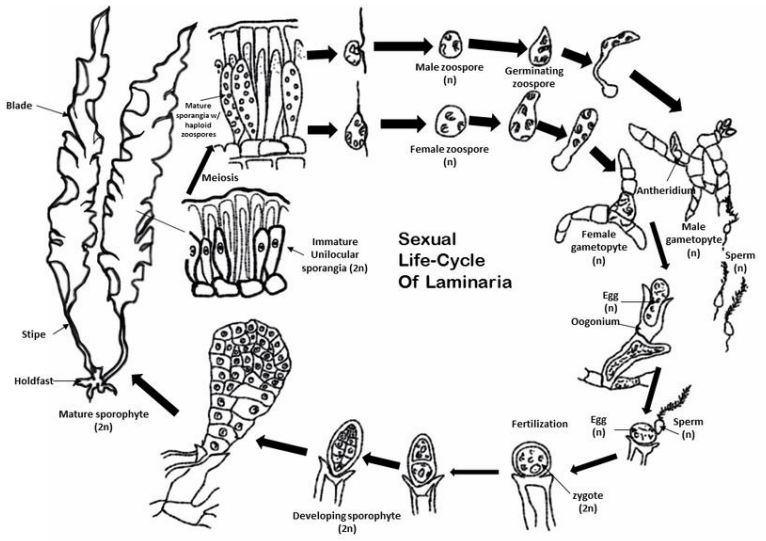


Figure 5.1 The life cycle of *Saccharina latissima* (and also other kelp species*).* Made by Morgan DeAngelis, April 2017.

5.1.1 Methods and materials

The cultivation of *S. latissima* and *A. esculenta* was done by two methods: 1) seeding on twine that are twisted around a coil, then nursed in the hatchery until the twine was attached on a thicker rope and deployed at sea, and 2) seeding of gametophytes/sporophytes on the final cultivation substrate using a binder to make them glue better to the rope, and then submerged in the seawater right after. Also, six types of substrate were tested to find the best growth substrate for the macroalgae. Substrate material used:

- 14 mm Polypropylene (PP) ropes, 3-twisted, with a 2mm diameter seeded twine
- Direct seeding of 12 mm diameter nylon ropes, braided (used from 2017)
- Direct seeding of AlgaeRope, braided BEXCO,16 mm
- Direct seeding of AlgaeRibbon T4 (5 cm), Breaking strength 2400 N/5 cm, patent-pending technology (W02015/087153).
- Direct seeding of AlgaeNets (2m x 10m), mess size 100 mm, patent-pending technology (W02015/087153)
- Direct seeding of AlgaeSheets (2m x 10m)

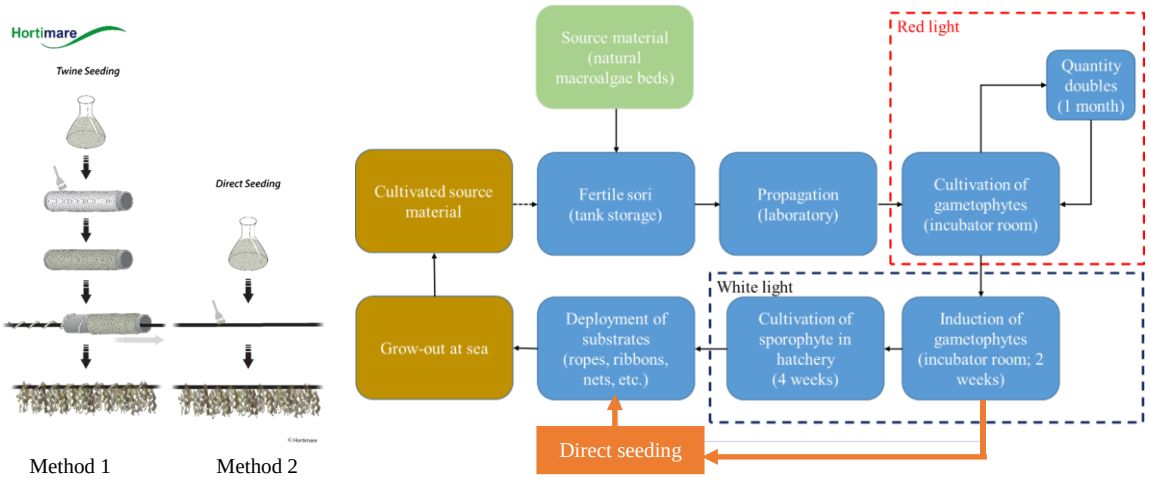


Figure 5.2 Method 1 and 2 illustrated by Hortimare **(left)** and the processes involved in seeding and the "short-cut" shown in orange when using method 2: direct seeding **(right)**.

Seeding method 1 follows the more traditional techniques, where spores are released in tanks filled with seawater and thin twine frames where spores can settle. This method requires a nursery period in hatchery/greenhouse. In the nursery the sporophytes are grown to a size of 1-2 mm to optimise their survivability at sea, and providing an advantage compared to natural seeding where high fouling rates occurs (**Figure 5.3e-f**).

The European adapted method uses an extra step where gametophytes are grown *in vitro* using red light to up-concentrate seeding material. In red light the gametophytes have vegetative grow and will not produce oogonia and spermatium. When enough gametophyte biomass is produced a period of white/blue light is used to induce fertility to start the sporophyte development. Hereafter, twines twisted around coils are seeded with the gametophyte/sporophytes soup and nursed on the coils under controlled conditions (Edwards and Watson 2011). After some weeks in the hatchery, the twine was twisted around a thicker rope and attached by cable ties for every 50-80 cm and finally deployed at sea.

Method 1 was used by Ocean Rainforest mainly in the years from 2013-2016 with some modifications. Method 1 was described in **PAPER I** and is illustrated in **Figure 5.3**.

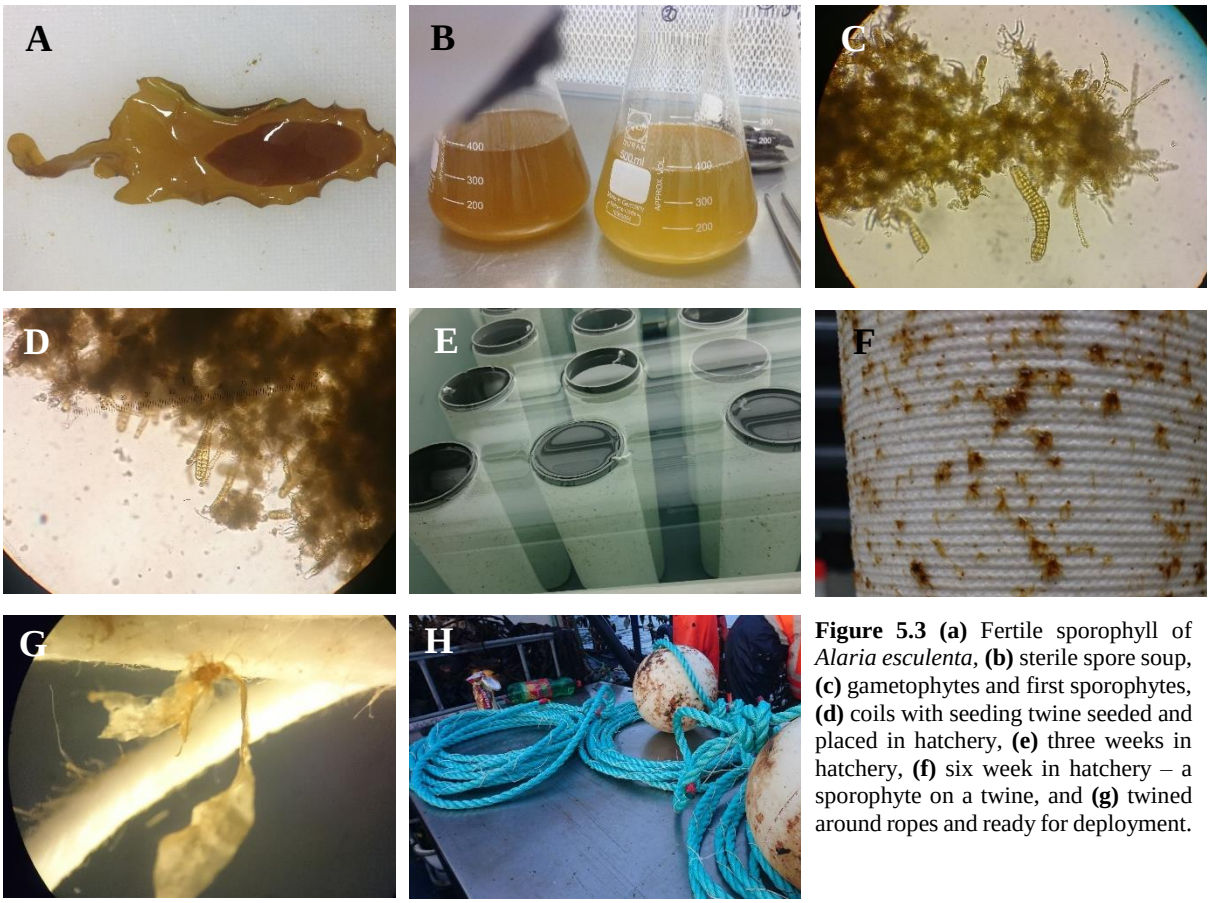


Figure 5.3 (a) Fertile sporophyll of *Alaria esculenta*, **(b)** sterile spore soup, **(c)** gametophytes and first sporophytes, **(d)** coils with seeding twine seeded and placed in hatchery, **(e)** three weeks in hatchery, **(f)** six week in hatchery – a sporophyte on a twine, and **(g)** twined around ropes and ready for deployment.

Method 2 followed the same steps as method 1 though a rope (or substrate) was directly seeded with the gametophyte/sporophyte biomass using a binder (Kerrison et al. 2018; **Figure 5.2** and **Figure 5.4**).

Figure 5.4 Direct seeding using newly developed sporophytes **(left)** and seeding on ribbons with a binder solution and squeezing out excess water **(right)**.

5.1.2 Results and discussion

Several seeding substrates were tested in order to find a substrate with high(est) yield performance.

A small experiment with three types of substrates was set up. The substrates were seeded with *S. latissima* and *A. esculenta* and monitored in June, July, August and November/December 2016 at the nearshore exposed site. *S. latissima* was deployed in May, two months later than *A. esculenta,* and data could therefore not be used for comparison among species. Also, the biomass of *S. latissima* was so low in June that measurement was not possible.

Using seeding the "twine around rope" method showed a density of more than 100 individuals for *A. esculenta,* in all monitored months except March and January (**Figure 5.5a**). Method 2 where *A. esculenta* was seeded on ribbons using a binder had slightly lower densities. *Saccharina latissima* seeded with the "twine around rope" method showed highest densities until November where ribbons, seeded after method 2, have similar densities (**Figure 5.5a**). Bexco ropes, seeding method 2, had the lowest density.

The density of *S. latissima* was markedly lower than *A. esculenta,* as a result of the two months later deployment and consequently a high fouling rate of green macroalgal species (most likely *Cladophora rupetris*), where the fouling coverage of juvenile *S. latissima* individuals was more severe/crucial. Density above 100 individuals per meter was not counted.

The longest individuals of *A. esculenta* (**Figure 5.5b**) were measured in June (~2 m), when seeded with the "twine around rope" method compared to ribbons (~1.5 m), seeded using direct seeding (method 2). The lines seeded with *S. latissima* had similar maximal length regardless of substrate type and seeding method. The longest individual was found in November (~1.5 m) (**Figure 5.5b**).

For *A. esculenta*, the interaction of month and substrate was significant (p=0.001). All months were different from each other except July and August when seeded on ribbons, and June and July, when seeded using method 1. In January the lines had no biomass but stipe and holdfast, hence lacking the meristematic zone (**Figure 5.5c**). Nevertheless, the *A. esculenta* did grow new blade directly from the stem in spring.

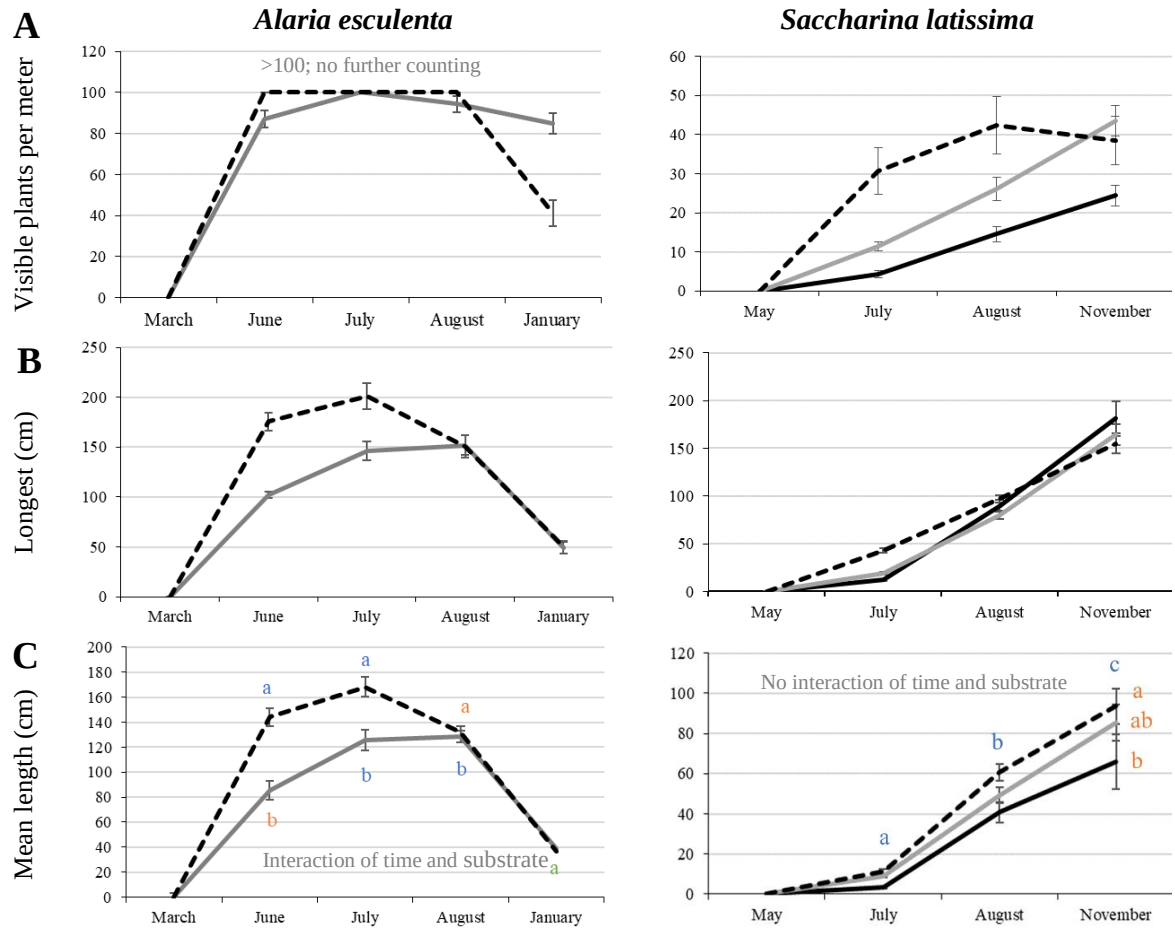


Figure 5.5 Mean ± SE of visible plants per meter (top), longest individual (mid) and mean length (lower) for *Alaria esculenta* **(left)** and *Saccharina latissima* **(right)**. *A. esculenta* lines were deployed in March 2016 and *S. latissima* lines were deployed in May 2016, at the nearshore exposed site, using three types of substrate: Bexco braided rope (black whole), ribbons 5 cm (grey whole), and twine around polypropylene rope (black broken line). The lines were monitored in June, July, August and November/January (n=3). Different letters on the graph represent statistical differences between substrates, for *A. esculenta* different colours of letters represent the statistical difference between months, for *S. latissima* different colours of letters represent variation between months (blue) and variation between substrates (orange).

For *S. latissima*, the blade length was increasing from deployment in May to last monitored in November. All months showed significantly different mean length (p=0.001). For *S. latissima* the longest individuals were observed for the "twine around rope" lines and on ribbons (method 2), and significantly longer than observed for Bexco ropes (p=0.001). Bexco ropes (method 2) and ribbons were not statistically different from each other (**Figure 5.5c**).

Ropes with the "twine around rope" method and ribbons directly seeded had thus a significantly higher growth length and higher density compared to Bexco ropes (method 2) for S. latissima.

For *A. esculenta* seeded substrates, the "twine around rope" method had higher growth compared to ribbons (method 2).

These results confirm that pre-nursed sporophytes, method 1, will kick-start growth and be in front compared to direct seeding of younger and smaller sporophytes, method 2. However, the seeding method "twine around rope" requires more steps and cost of the hatchery/nursery phase and these extra steps can be significant compared to the "short cut" using the direct seeding method (**Figure 5.2**).

A three-twisted polypropylene (PP) rope, 14mm in diameter, has a price of €0.40 per meter and the twine, 2mm in diameter, used in hatchery has a price of €0.53 per meter, a braided nylon rope, 10mm in diameter, has a price of €1.21 per meter, and special designed seeding substrates like ribbons, 50mm wide, and Bexco ropes, 14mm in diameter, from "AT~Sea Technologies" have a price of €1.61 per meter. To use the more expensive substrates the yield should consequently be equally higher (**Table 5.1**).

Unfortunately, no valid yield or length data was made for nylon ropes directly seeded, and the final comparison between all substrates and seeding methods cannot be fully made. Also, the yield instead of length would have help in this conclusion.

However, when using the results available to compare profit, the seeding method with "twine around rope" has the highest yield (length) for *A. esculenta* and this seems to thump the otherwise lower total seeding cost for ribbons, Bexco ropes and nylon ropes when seeding this species.

For *S. latissima* the results proved that direct seeding can be used equally efficient as seeding the method "twine around rope" when compared among lengths. Beside the length and cost results for direct seeding, the method is preferred compared to using polypropylene rope and twine, as the risk of cable ties that is used to tie twine to rope represent a major risk during harvest in terms of cable ties being cut of and mixed with the biomass. Thereby a food hazard as a foreign object made of plastic.

A similar substrate and seeding comparison was made by Kerrison et al. (2018). They found that the life stage of the seeding material used for direct seeding had a major impact on the

results, for example, resulted sporophyte seeding in a twice as high final biomass yield compared with gametophyte seeding due to a two to three weeks developmental lag, while meiospores-seeding (few days old) gave very poor results. Furthermore, they concluded that the direct technique, using a binder (method 2), is an effective method to allow textile substrates to be seeded, and the method, when using sporophyte life stage, gave an equivalent final biomass to when grown within a traditional hatchery. However, they conclude that the method needs further optimisation and testing to ensure its reliability.

Table 5.1 The total cost of seeding and deploying growth lines on a Macroalgal Cultivation Rig (not including costs related to the installation of MACR). The total number of growth lines were set to 250 given a total of 2500 m growth line. The length obtained on the tested substrates was presented in **Figure 5.5**. Red numbers indicate the decrease in length among substrates, where PP ropes had the longest mean length. Seeding method 1 = includes a hatchery phase, seeding method 2 = direct seeding. PP = polypropylene.

	Seeding method 1	*Seeding method 2*		
Substrate	**String on PP rope**	**Ribbons**	**Bexco**	**Nylon**
Unit	€	€	€	€
Seeding material (cost/m)	1.88	1.88	1.88	1.88
Seeding material (sporophytes; 2500m)	4700	4700	4700	4700
Substrate (cost/m)	0.4	1.61	1.61	1.21
Substrate (2500m)	1000	4025	4025	3025
Coils	80	-	-	-
String (twine)	1340	-	-	-
Hatchery container	1005	-	-	-
Labour under nursing	1005	-	-	-
Cable ties	250			
Labour to twine string around rope	1150	-	-	-
Labour direct seeding	-	435	435	435
Deployment	1065	1065	1065	1065
In total seeding cost per MACR	**10530**	**9160**	**9160**	**8160**
Cost reduction	-	**13%**	**13%**	**23%**
Alaria esculenta (length July)	165	125		
Length difference (*A. esculenta*)	-	**-24%**		
Saccharina latissima (length Nov.)	93	85	65	
Length difference (*S. latissima*)	-	**-9%**	**-30%**	

Additionally, the ribbons turned out to have a lower breaking strength (*21kN/5 cm*) than needed under operation and handling at a MACR-RUNI structure. This structure requires that the fixed line can be lifted above the surface, by lifting a growth line, using the crane. Under this

operation, the ribbons brook frequently, and they did also break during storms after being deployed at sea over a 1-2 years period.

In perspective of more sustainable substrates second-hand fishing ropes were directly seeded and deployed at sea. The second-hand ropes had normal growth (data not shown) and can be used in future operation if available. Currently used fishing ropes ends up in huge bundles that are challenging to untangle.

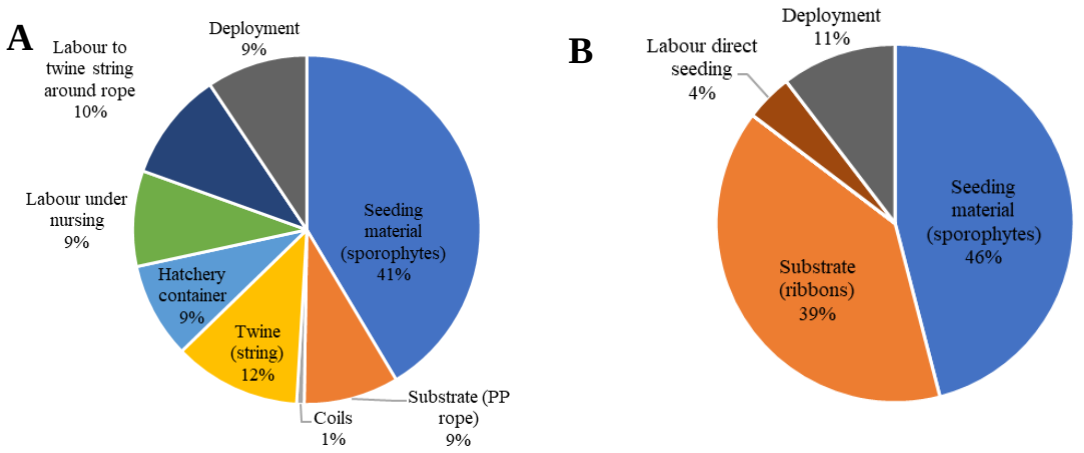


Figure 5.6 Cost as percentages of total cost per meter (not including the cost of rig installation). **(a)** Polypropylene ropes with twine seeded and nursed in hatchery had a total cost of €4.1, and **(b)** ribbon direct seeded with a total cost per meter of €3.6.

Finally, an experiment tested nets and textile sheets on the nearshore exposed cultivation site. These were direct seeded and attached to the fixed line. Within few months, the 2D-substrates came loose and were lost from the cultivation structure. This underlines that macroalgal cultivation sites in nearshore exposed or offshore sites cannot use nets and sheets, due to strong drag forces. However, these substrates could be relevant to use in more sheltered sites.

5.2 Seeding of *Palmaria palmata*

The red macroalgal species *Palmaria palmata* is commonly known as dulse can be found on each side of the Atlantic Ocean. Wild stock of *P. palmata* has a long history of human utilization and is one of the most important edible macroalgae, which has been used for more than 100 years (Titlyanov et al. 2006). *Palmaria palmata* is collected by hand picking at low tide in eastern Canada, Iceland, Ireland and in Nova Scotia (Garbary et al. 2012). The species has a

great market interest because of its high protein content of up to 35% (Fleurence 1999), and the demand for *P. palmata* as a snack food regularly outstrips the supply obtained from naturally growing populations.

For a stable supply and to meet the demand, cultivation trials were initiated in France (Le Gall et al. 2004), and further developed in Northern Ireland at Queen's University Belfast, at the Marine Laboratory (Edwards 2008, Edwards and Dring 2011, Astrid Werner and Dring 2011a). More recently, the search for novel blue biomass has intensified the work and many European research projects are today aiming the commercial cultivation of *P. palmata*. The price of *P. palmata* is today at a price of €50/kg dw when sold to the food market. Many macroalgal farmers would, therefore, endeavour to grow it.

In the Faroe Islands, *P. palmata* has a wide distribution along the coastline in the intertidal zone. Often it sits where there is freshwater run-off and shading. Further out from the rocky coastline, *P. palmata* sits on the stipe of the large brown kelp species *Laminaria hyperborea* and *Laminaria digitata*. In countries like Canada, Ireland and Iceland, the advances of large tidal differences and a gentle slope are important compared to Faroese conditions. Here the slope is steep, and the tidal differences are small (1-2 m). Even though *P. palmata* is abundant, the utilization of this wild population is challenging. To collect them, the possibilities are hand-picking from the stipe of *Laminaria* sp. using divers, or a person on land who are willing to take the risk on the slippery rocks between hitting waves. The natural collecting of wild populations was therefore not attractive for Ocean Rainforest, labour intensive, "risky" business, and would also not be a sustainable utilization because natural alga beds would be disturbed.

In order to grasp the major challenges experienced during the cultivation trials, it is necessary to understand the life cycle of *P. palmata*. The next section therefore briefly explains the life cycle. The following chapters describes different approaches for cultivation of female sporophytes, natural seeding techniques and controlled tetraspore release. Some of the experiments were more successful than others, but the work that was made does certainly deserve attention. Each approach begins with a brief materials and methods description and are followed by the results and discussion of these before introducing the next approach.

5.2.1 The life cycle of *Palmaria palmata*

The life cycle of *P. palmata* was first understood in 1980 by van der Meer and Todd. The life history of this alga has before that investigation been of high interest for phycologists, because the female gametophytes was a mystery. What is also special about *P. palmata* is that the male gametophytes (1n) has similar size and morphology as the tetrasporophyte (2n) (van der Meer and Todd 1980), which complicate the discrimination of these. The tetrasporophyte releases tetraspores (n) (spores clearly divided in four) and these passively settle and develop to a male and a female gametophyte. The male (n) develops, as mentioned, into a fleshy macroalgae, that when mature releases spermatia, whereas the female (n) grows not more than into a dwarf/crustose morphology, and carries the carpogonium inside, but has a long trichogyne. That reaches out to catch the spermatia. The male gamete (n)(spermatia) has no flagella, which reduces the chances of swimming towards the female gamete for successful fertilization in an environment that dilutes and moves everything. However, by successful fertilisation, if a spermatia has been caught by the trichogyne, the spermatia are transported to the carpogonium, and this results in a fertilised zygote, the tetrasporophyte (2n) that starts to cell divide and grow from the crustose remains of the female.

The crustose microscopic female gametophyte (n) is less than a millimetre in diameter, while the male gametophytes and the tetrasporophytes can reach 30-50 cm in length. The female gametophyte becomes fertile within a few weeks, whereas the male gametophyte needs an 8-12 month of growth before getting reproductive. This “unbalanced” partnership make fertilization very difficult under cultivating as eventually half of the females will not develop into harvestable biomass as they are not fertilized. Consequently, precociously fertile females are almost certainly unable to mate with males of the same gamete-generation and are most probably fertilized by older males from the preceding generations that have survived the winter.

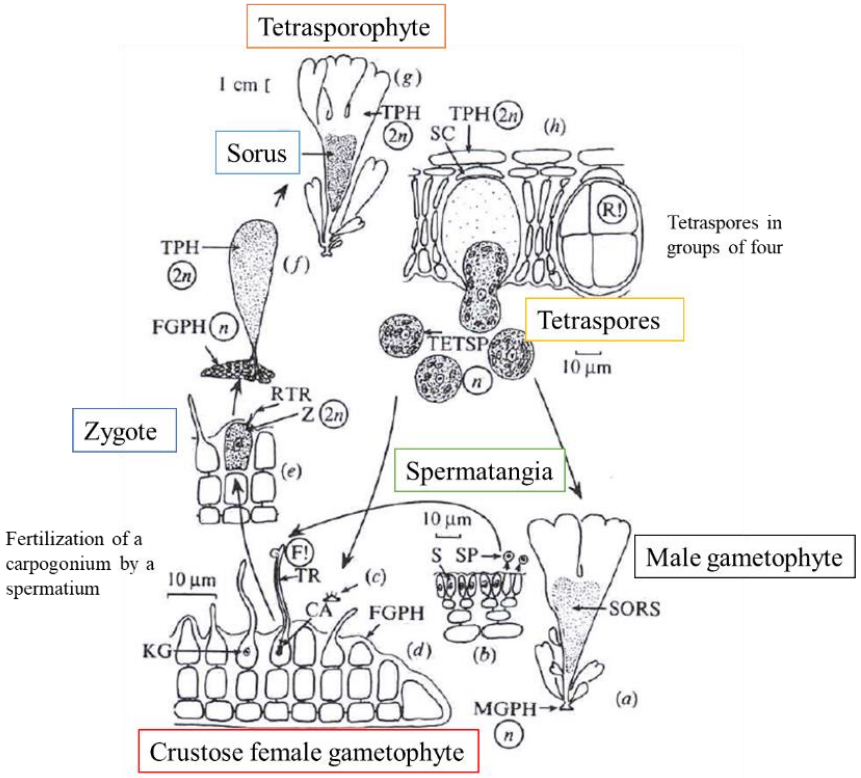


Figure 5.7 The life cycle of *Palmaria palmata.* The drawing was copied from Werner and Dring (2011b) who refers to the findings of van den Hoek (1995). The figure is slightly modified as important names for a better understanding of the life cycle are highlighted with a coloured frame. Not all figure names are explained here. For the full explanation see Werner and Dring (2011b).

The important work by van der Meer and Todd (van der Meer and Todd 1980) finally proved the existence of female gametophytes which was otherwise questioned.

5.2.2 Vegetative growth used for cultivation at sea

Palmaria palmata can be cultivated by vegetative reproduction, and at sea cultivation was tempting using the MacroAlgal Cultivation Rig (MACR) (section 3.2.1 "Cultivation method and optimisation").

To test growth at sea, *P. palmata* individuals were collected and pieces of 5-10 cm were inserted into the line for every 15 cm of a 10 m long rope **Figure 5.8**. This could be done by "untwisting" the rope at a spot, inserting some of the basal part of the thallus and straitening the rope again. The pull at each end of the rope would keep the thalli trapped. Three ropes were deployed as a trial at the nearshore exposed site in Funningsfjørður in March 2016, and for a four months period, the pieces had grown to a size of 30-50 cm (**Figure 5.8**). Some of the inserted pieces were, however, lost because of the strong oceanic currents and the weak attachment gained through this method.

Figure 5.8 Pieces of *Palmaria palmata* inserted on a three-twisted rope **(left)** and full-grown individuals of 30-50 cm length at sea **(right)**.

While the good growth results showed that *P. palmata* could grow to large biomass at the exposed site, the ropes should still be seeded denser to make it commercially interesting. The sexual seeding techniques developed in France (Le Gall et al. 2004) and Northern Ireland (Edwards 2008, Edwards and Dring 2011, Astrid Werner and Dring 2011a) were therefore proposed and tested.

5.2.3 Seeding on twine using fertile tetrasporophytes

Numerous trials were conducted in order to optimise the techniques for obtaining spores of *P. palmata*, for an optimal settling and the maintenance in the hatchery until they were ready for transfer to the sea for out-growth. The techniques summarised here represent the conclusion of these trials and in the coming sections some alternative strategies.

Seeding onto twines using fertile *P. palmata* tetraspores was described before by Edward & Dring and Pang & Lüning and also the out-planting at sea was tested before in small scale (Pang and Lüning 2004, 2006, Edwards 2008, Edwards and Dring 2011, Astrid Werner and Dring 2011c). The advantages of this method are the sexual reproduction that enables a full seeding of the cultivation substrate. Following these protocols, a series of seeding trails were performed; though, the seeding was not easily done.

Mature *P. palmata* was collected in winter months from wild populations in Tórshavn, Tjørnuvík, and Funningsfjørður, in the Faroe Islands. The experiments were made in the laboratory and hatchery facilities in Kaldbak. The collected algal material was kept in seawater at a temperature of 10-12 °C with aeration, low light (<40 μmol photons s^{-1} m^{-2}) and daylength (6:18 day:night regime) reflecting the season until the biomass was used in seeding trials.

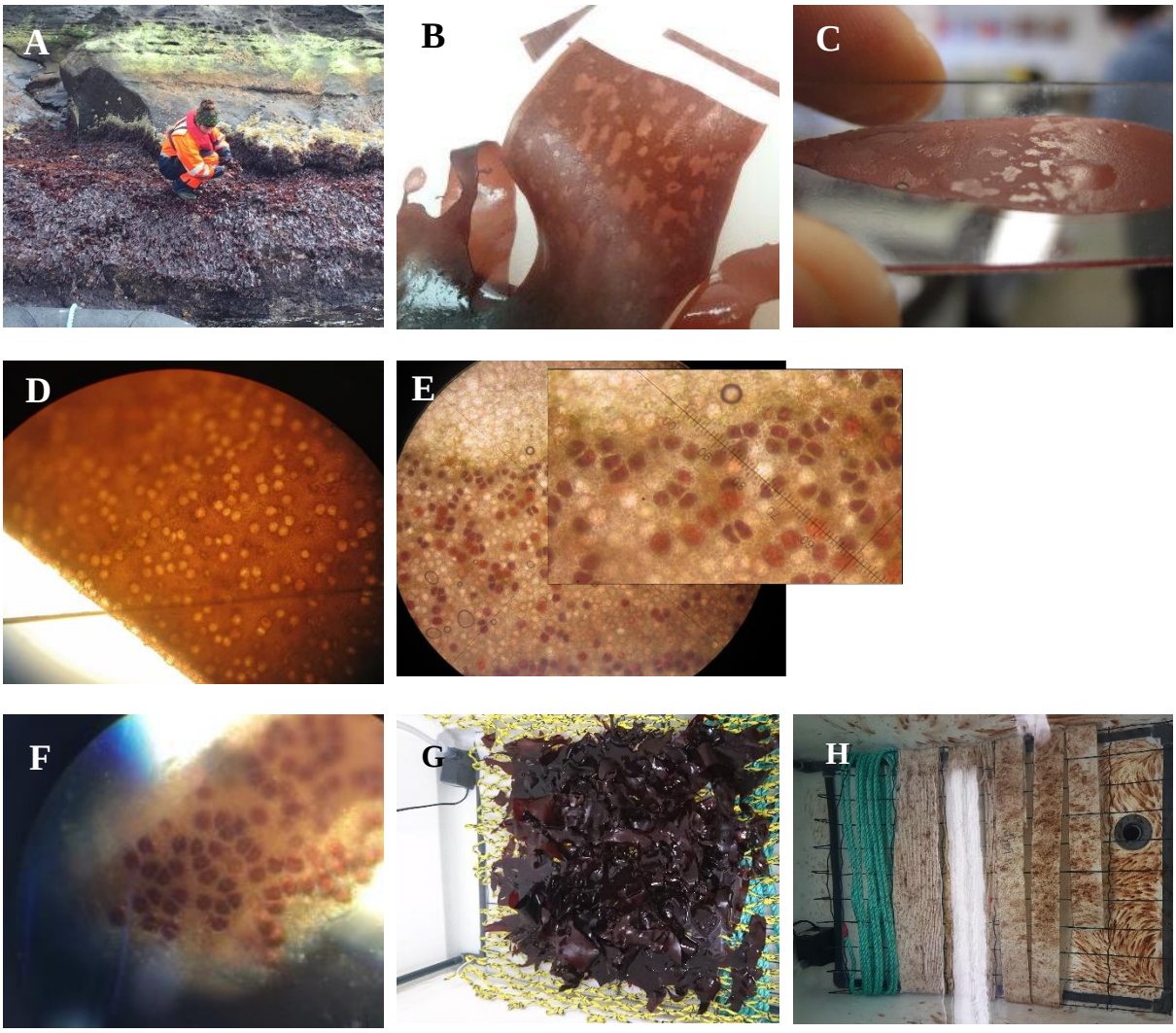


Figure 5.9 (a) The Faroese shoreline covered by *Palmaria palmata*, **(b)** and **(c)** the fertile part of a blade with darker red areas than the remaining front, **(d)** a tetrasporophyte with mostly empty tetrasporangier under microscope (magnification x100), **(e)** and **(f)** mature tetrasporophyte with spore arranged in pairs of four (magnification x100 and x200), **(g)** fertile fronts placed in a net above the substrate for 4 days, and **(h)** no sign of *P. palmata* on the substrate (several types) but fouling of filamentous brown algae.

Spore release of *P. palmata* was made according to the method described by Werner and Dring (2011a), using individuals with visible dark red areas of sorus (**Figure 5.9**). Some of the experiments used the approach of Le Gall et al. (2004), where dehydration overnight is included after cleaning procedure similar to the method used for kelps. Some of the experiments used also chlorine sterilisation, though without success. Fronds with visible epiphytes or endophytes were removed and discarded to avoid contamination. The tetrasporophyte and male gametophyte can be difficult to distinguish by eye, and male gametophytes might also have been present.

Though the experiments were made according to protocol, they were never successfully, and most useful results were found when least excepted. Before we go more into details with the unexpected result, we need to focus a bit on what went wrong and what the challenges are for the previously described. There was a high fouling of brown filamentous algae and no visible tetraspores. Several substrates were tested, though this didn't help the absence of tetraspores. The fronts were all fertile but did not release tetrasporophytes according to the protocol.

5.2.4 Sterilisation and prevention of fouling and epiphytes (contamination issues)

One major concern when cultivating *P. palmata* was the control of contamination, as fouling an epiphyte can inhibit the growth of the young gametes. A chlorine sterilisation method is often used for kelps. Hence was the chlorine method tested on *P. palmata*.

Seven different concentrations of chlorine were tested using not fertile *P. palmata* fronts to get closer to a concentration that could be suitable. Each front was cleaned in a chloride bath for 1 min followed by two seawater baths. The solutions were made from sterile seawater and 15% Sodium Hypochlorite (NaOCl) in seven concentrations: 1.2%, 0.6%, 0.1, 0.05, 0.025, 0.01% and no treatment. After the three baths, they were followed in up to 12 days placed in Erlenmeyer flasks with sterilised seawater, aerated and had 12:12 day:night regime and weekly water exchange. Triplicates fronts were placed in each Erlenmeyer flask and triplicates of each concentration were made.

The tissue was inspected by microscope (magnification x200) for contamination and cell-depth was inspected as the colour of tissue, with the red colour giving the highest score and white, green or brown tissue giving a lower score.

Unfortunately, a 1.2% chlorine solution was too concentrated and fatal as fronts turned white-brownish and degraded. 0.6% had no significant effect compared to the control. Contamination was observed in all lower treatments from day two and in the control. The conclusion was, therefore, to use 0.6% - though this was not tested for mature fronds.

Silje Forbord (SINTEF, pers. comm.) made similar sterilisation experiments with NaOCl and concluded that chlorine sterilisation was not suitable and that other disinfection methods should be tested e.g. lugol or acidic acid. Eleanor Woods (SAMS, pers. comm.) found good results with a solution of Potassium Iodide (KI) 0.5% as used for 10 min. Hanic and Pringle (1978) used ethyl alcohol (l00 %) to sterilise *Chondrus crispus* before spore release, by having fragments with mature sori dipped into ethyl alcohol for 1-2 seconds. Other methods could be iodine treatment, but this has to our knowledge not yet been tested.

Alternatives to sterilisation would be the cleaning method by Pang and Lüning (2006) using sterile seawater and repeating the cleaning procedure three times and changing water three times, and then when cultured adding germanium dioxide (GeO_2) to avoid diatoms contamination.

5.2.5 Induction of maturity

Often, and mainly during summer, it is difficult to find fertile fronts. For a larger production scenario, it would be optimal to control the life-cycle and induce fertility out of season.

Pang and Lüning (2006) described a method to induce mature tetraspores by stimulating winter over a two and a half months. This is, however, a relatively slow process.

In this work, freezing was tested to induce mature sori. Samples were frozen for 2, 4 and 24 hours, and cultivated in seawater subsequently. Sadly, none of these freezing times resulted in fertile tissue. This result was also supported by Silje Forbord (SINTEF, pers. comm.) who made a similar freezing experiment.

5.2.6 Seeding by accident – a good strategy?

The twine seeding trials did not result in spore release; however, excess biomass from the same harvest was stored in tanks with gentle aeration, low light and short-day conditions, just for having biomass in case of new experiments were needed. The tanks of 200 L had frequent water changes (1-2 days). After two weeks, spontaneous spore release was observed in the tanks. This was clearly observed by eye on the white tank bottom, but also observed in a water sample under a microscope. Unfortunately, the sporulating tissue was not sterilised (as these were not expected to have spore release) and the seeding with the tetraspores after spore release was under high contamination conditions.

The tetrasporophytes were removed from the tanks, which was slowly emptied for water ensuring that the tetraspores stayed on the tank bottom. Hereafter, the tetraspores was seeded on twines and ribbons by scrubbing of the tank bottom and sides. The seeded substrates were then placed in a clean tank filled with filtrated and UV-treated seawater and nursed for two-three months before deployed at sea.

Inspecting the lines after a period confirmed the growth of the male gametophytes on the seeded substrates, though with a very low density. Unfortunately, the sporulating tissue was not sterilised (as these were not expected to have spore release) and the seeding with the tetraspores after spore release was under high contamination conditions, which leading to a high competition between fouling and the seeded *P. palmata*.

The good news was that *P. palmata* when brought to the laboratory under low light and short day length could, if already fertile, within few weeks make spontaneous spore release as described by Pang and Lüning (2006). The fact that tetraspores can be collected after they have settled to the tank bottom suggests new possibilities for commercialising this – if the sporophytes previously to entering the tank are sterilised and if tank and seawater are auxenic.

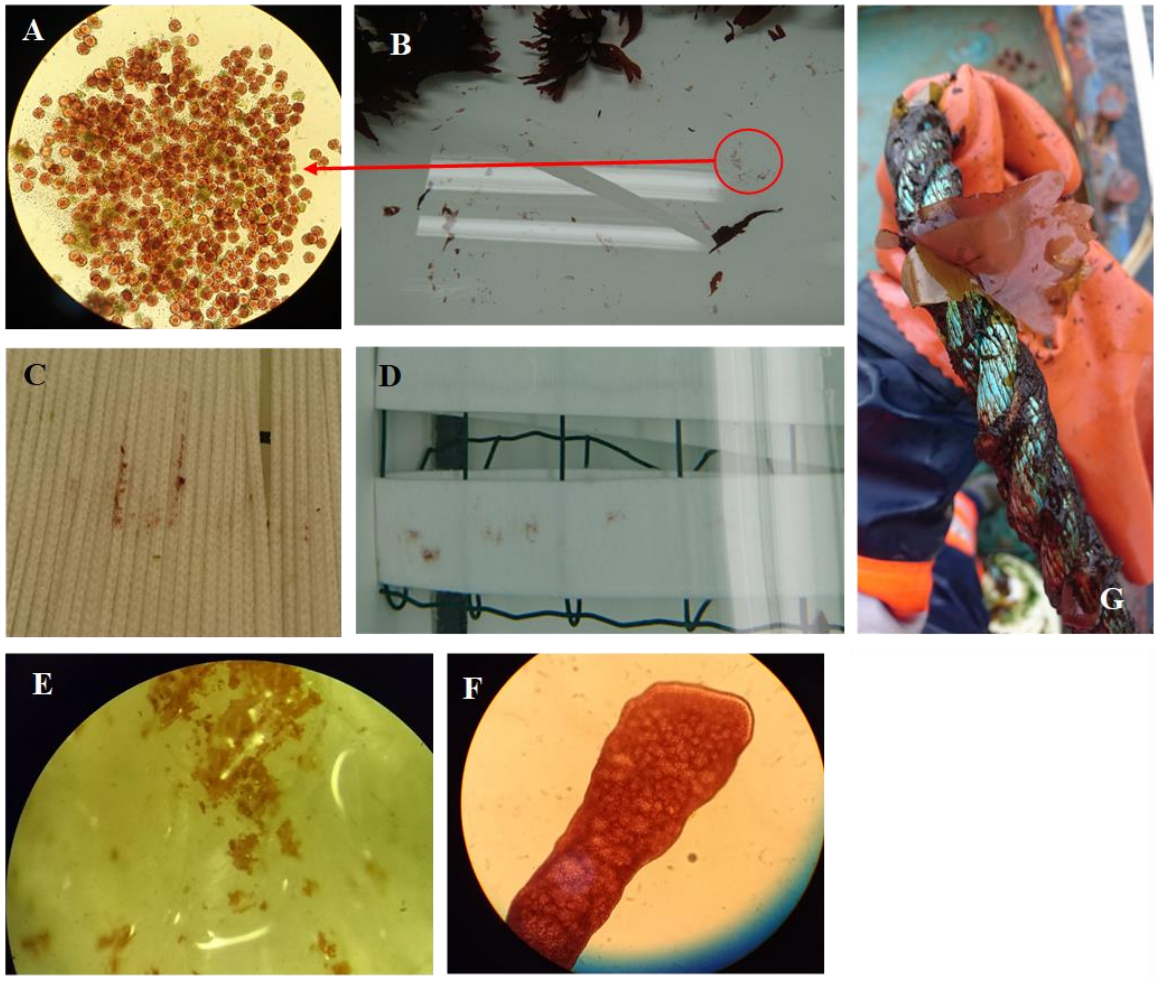


Figure 5.10 (a) *Palmaria palmata* tetraspores under microscope under not-sterile conditions (magnification x200), **(b)** mature *P. palmata* tetrasporophytes and tetraspores released in a white tank and observed by eye as red patterns on bottom, **(c)** gametes growing on seeding twines in hatchery, **(d)** gametes growing on ribbons in hatchery, **(e)** few days old gametes on twine under microscope (magnification x100), **(f)** a male gametophyte after two months in hatchery previous to deployment at sea (magnification x100), and **(g)** *P. palmata* growing at sea.

5.2.7 Direct seeding of *Palmaria palmata*

Palmaria palmata collected on February 26, 2016, was stored in seawater at high density (approximately 200 g biomass per litre) in small plastic tanks of 20 L. After 11-14 days with low aeration, daily water change, temperatures at 10°C and natural day light (short days low light through roof window) the seawater turned pink and tetraspores were observed in a water sample inspected under the microscope, though also high mortality was found among tetraspores.

Five ribbons of 10 m length were direct seeded using the pink water. The pink water was mixed with the previous mentioned binder/glue and the ribbons were submerged in the pink-glue-tetraspore solution. The excess seeding liquid was squeezed out of the ribbon manually.

The ribbons were deployed at sea at the exposed cultivation site (A71) on the following day (March 9, 2016). The lines were inspected regularly, and visible male gametophytes were observed in January 2017. The growth on the substrate of *P. palmata* could not be due to natural self-seeding as control "ribbons" with no seeding, were deployed at the same time as the seeded, but the control lines had no growth of *P. palmata.*

The pink water could be a result of oxygen depression as the boxes had a high density of *P. palmata*. Together with the identified tetraspores, the accessory pigments of red colour might also have been released colouring the water, due to stress from the anaerobic conditions. These conditions might have killed or affected some of the tetraspores resulting in low density. However, pigments and accessory pigments are bound in the thylakoids together with the chlorophylls, and the thylakoids are located inside the algae cells, so the stress should then have opened/leaked the cells.

This successful direct seeding of *P. palmata* was to our knowledge proved for the first time.

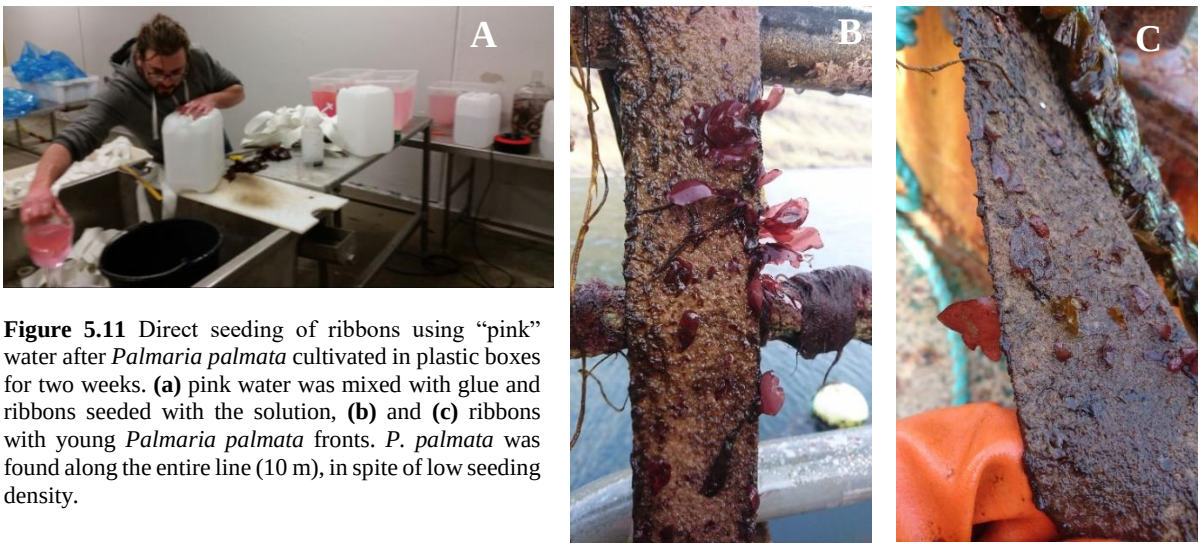


Figure 5.11 Direct seeding of ribbons using "pink" water after *Palmaria palmata* cultivated in plastic boxes for two weeks. **(a)** pink water was mixed with glue and ribbons seeded with the solution, **(b)** and **(c)** ribbons with young *Palmaria palmata* fronts. *P. palmata* was found along the entire line (10 m), in spite of low seeding density.

5.2.8 Perspectives of fertilised females, and cultivation and upscaling these

The low seeding density is a major challenge to the commercialisation of *P. palmata*. Recently, Peter Schmedes, DTU Aqua/NTNU, has suggested to co-cultivate fertile male gametophytes with newly released tetraspores. In method simulate what happens in nature, as older male gametophytes (> 8 month) fertilize the next-generation of female gametophytes, and instead of having tetraspore-seeding with only male gametophytes growing to a harvestable biomass, also the female gametophytes are being fertilised and the zygotes grow into harvestable sporophytes biomass side-by-side with the male gametophytes, and thus doubling the density. This innovation is a step in the right direction, though seeding biomass upscaling is still not developed.

To grow female gametophytes has until recently been a "no go" as these were described to have their mature life period in weeks after tetraspores release where they produce trichogynes within a few days of existence. If not fertilised, the number of trichogynes declines and they become rare on females 4-5 months old, which by then have grown into small, misshapen and stunted organisms and they would die within few weeks (Werner and Dring 2011a).

After one of the *P. palmata* spore-releases, in this present work, a strong red coloured growth-out was observed on one of the lines. From curiosity, it was placed in a flask with aeration and nursed (with nutrients, water exchange, light, etc.). This unregular red dot was very different from "normal tetraspores" (**Figure 5.12**) and was suspected to be a female gametophyte, as it also developed long trichogyne. In comparison with figures of female gametophytes shown in van der Meer and Tood (1980) and Werner and Dring (A. Werner and Dring 2011a), our replicate looked very similar.

Surprisingly, the suspected female gametophyte survived more than eight months. The gametophyte was not nursed sufficiently to the end, and finally fouling and contamination overgrew it.

Female and male gametophytes of kelps are capable of vegetative growth after mechanically being divided, and thus increasing seeding material significantly. The hypothesis of Phillip Kerrison is therefore that this will be possible to the *P. palmata* females as well.

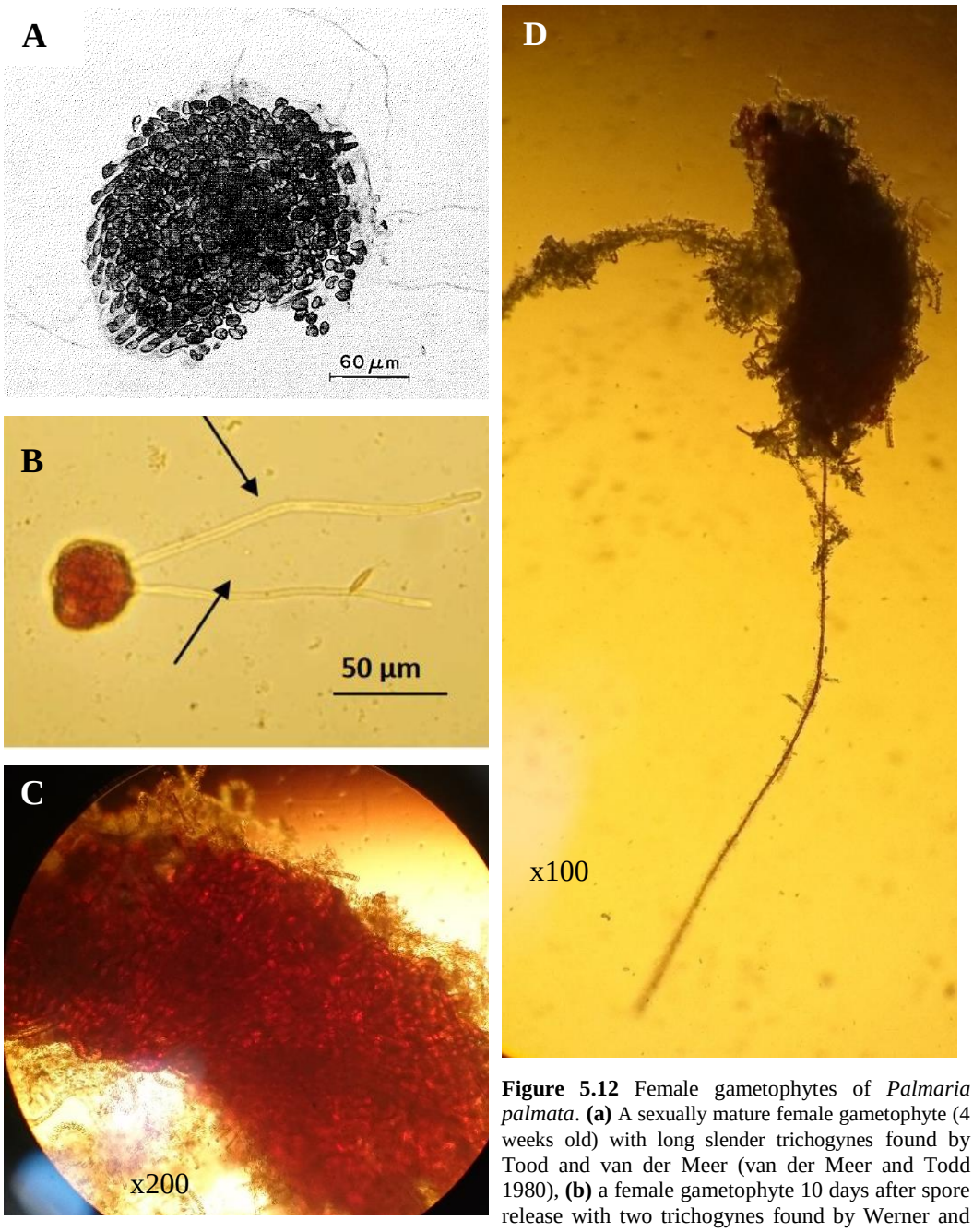


Figure 5.12 Female gametophytes of *Palmaria palmata*. **(a)** A sexually mature female gametophyte (4 weeks old) with long slender trichogynes found by Tood and van der Meer (van der Meer and Todd 1980), **(b)** a female gametophyte 10 days after spore release with two trichogynes found by Werner and Dring (Werner and Dring 2011b), **(c)** a female gametophyte (8 months old) cultivated in Kaldbak, Faroe Islands, and **(d)** the same female gametophyte in a lower magnification showing the long trichogynes even after 8 month of cultivation.

These findings and the possibilities for new seeding methods strongly support that cultivation of *P. palmata* can be possible in near future, also in large scale.

5.2.9 Natural seeding experiment

The cost and time consumption of producing *P. palmata* seeding material is high when considering collecting material in the field, laboratory work and expenses related to equipment and hatchery. This was solved by a single test made during winter 2017-2018.

In November 2017, three nets made from standardly used fishing nets (nylon; diameter 5mm) with a size of 1x1m and mesh size of 2x2 cm were fastened to the rocks in Funningsfjørður. The nets were placed upon natural patches of *P. palmata* nailed into the rock.

In May 2018, these net were perfectly seeded with *P. palmata*. The nets had no fouling, except from some *Pyropia* sp. (formerly known as *Porphyra* sp.). Each of the nets had a yield of 5 kg ww and the length of the *P. palmata* was 1-5 cm. The natural seeding methods have some limitations:

- Considering large scale production, the natural population ”spots” of *P. palmata* along the coastline will be limited, and the use of these spots will affect the natural habitats.
- The rocks are slippery and it is not easy to work in these areas.

The seeded nets were brought to the MacroAlgal Cultivation Rig at the nearshore sheltered site and fixed to the fix-line of a MACR-DIA. Here they were left for further out-growth to increase yield. In July the nets were inspected. To our disappointment, the nets had decreased in yield to approximately 1 kg ww per net compared to when deployed two month earlier. Grassing could be a explanation, but also the lack of tidal exposure may have high influence.

Extrapolation made by Sanderson et al. (2012) showed that under optimal conditions, a one-hectare cultivation site seeded with *P. palmata* could yield up to 180 tonnes ww per annum. One MACR of 500 m takes up the space of one-hectare sea area. If a cultivation rig of the type MACR-DIA had nets of 1 m^2 size hanging vertically and continually to its horizontal fix-line floating 0.5m below main water level it would amount to 500 m^2 of cultivation substrate. If each m^2 yields 5 kg ww when deployed and yield approximately 50 kg ww of harvested material per year, then this would correlate to 25 tonnes ww/ha, or approximately 2.5 tonnes dw/ha. This could be sold at a price of €50per kg dw and the gross income would then be € 125.000/ha.

Furthermore, the question about multiple partial harvesting has to be addressed – it will have a significant impact on capital expenditures (CAPEX) cost reduction if the same m^2 can be harvest for example over three years without reseeding.

However, the successful seeding with this method requires further trials to obtain a successful growth cycles at sea. Several aspects need to be investigated, for example, if the seeded *P. palmata* substrates will have a higher survival rate if the nets are placed horizontally in the water column compared to vertically. Another idea could be if the cultivation rig is fixed to the seafloor allowing the substrates to be exposed to air in low tide (and thereby replicating the growth conditions in natural beds of *P. palmata*). Such research will also need proper monitoring of environmental conditions (current, salinity, wave, light) in order to confirm the potential correlation between yield, cultivation method and location.

Time of deployment was investigated as well to make the cultivation more flexible and to test best growth performance. *Saccharina latissima* and *A. esculenta* was deployed in most months of a year, covering also spring and summer, and showed that growth was observed after all deployments, but that the May deployment had lower density and lower yield compared to deployments in other months. Deployment during spring or summer were therefore not advisable, and for optimal growth, the deployment in autumn is still recommended - even though the sea temperature in the Faroe Islands is stable year-round at 6-11 °C.

The work aimed also to seed the red macroalgae species that is not cultivated at sea today. The spore release of *Palmaria palmata* was very difficult to control, and low light, lack of water flow or freezing did not help. However, spore release was obtained from mature tetraspores when cultured in tanks under winter conditions (low light and short days) and was used to direct seed *P. palmata* for the first time.

Natural, seeding of nets placed in *P. palmata* populations had high density and growth of *P. palmata.* When the nets deployed at sea, the biomass decreased. More research is a need for defining the best cultivation conditions for this species. Fixed structures enabling tidal exposure might be needed for exploiting full growth potential of this intertidal species.

Figure 5.13 Natural seeding with *Palmaria palmata* on nets attached to the shore in Funningsfjørður. **(a)** Nets nailed on the rock in November 2017, **(b)** nets de-attached from rock in May 2018, **(c)** nets being prepared for deployment on the MacroAlgal Cultivation Rig, **(d)** a close-up picture of the nets on the front side, **(e)** a close-up picture of the nets backside, and **(f)** the nets holding 5 kg wet weight/m^2.

6 Conclusion and perspectivation

6.1 Conclusion

One of the main objectives of this PhD study was to obtain in-depth knowledge about the biochemical composition of the commercially attractive macroalgal species *Saccharina latissima, Alaria esculenta* and *Laminaria digitata*. The objective was also to investigate growth at sea and optimize yield of the cultivated macroalgae when cultivated in open-ocean. The work had furthermore the objective to investigate optimal seeding techniques for *S. latissima* and *A. esculenta* from a commercial perspective. Finally, this work aimed to investigate and optimise induction methods for seeding of the red macroalgal species *Palmaria palmata*, to test trigging parameters for controlled spore release and up-concentration of seeding material for commercial production.

Regarding the seeding experiments, research resulted in many lessons learned and new innovative approaches tested. The direct seeding of ribbons showed promising results, as the method was cheaper than the more traditional "twine around rope" method, and for *S. latissima*, equal biomass (length) were observed between the two methods and substrates. Direct seeding on nylon ropes was tested on commercial scale from 2017, but not monitored sufficiently to compare the viability of this substrate with the other substrates. Nevertheless, the commercial deployments of directly seeded nylon ropes showed that *S. latissima* can grow sound on this cheaper substrate. Our hypothesis was therefore confirmed as direct seeding can compete with the more traditional methods with regard to length growth, and this method is also cheaper.

Controlled spore release for *Palmaria palmata* was tested using low light conditions, no water flow and freezing, but with no success, and thus was our hypothesis rejected (H3.2). Instead "spontaneous" spore release was observed by bringing fertile tissue into semi-controlled tank facilities. The tetraspores could subsequently be collected on substrates, hereafter directly seeded and deployed at sea or grown in the hatchery until later deployment. The growth-out at sea was not promising as a low *P. palmata* density and high fouling of other macroalgal species were observed.

A new seeding method was tested, using "natural seeding". Nets were placed on rocks covered by *P. palmata* in autumn and in spring the nets revealed a high density and growth of *P. palmata.* When the nets were then moved and deployed at sea, the biomass decreased significantly over a period of two months. Our hypothesis that natural seeding of nets is possible when placed at sites with wild populations was therefore confirmed (H3.3), but more research is needed to be able to define the best cultivation conditions for this species at sea. A fixed structure is proposed as further testing and research, since this would enable tidal exposure and may show the full growth potential of this intertidal species.

Furthermore, a female *P. palmata* gametophyte was cultivated *in vitro* for more than eight months, which strongly supports a new hypothesis by Phillip Kerrison and colleagues at SAMS in the UK, that females can be cultivated and may be capable of vegetative growth after mechanically being divided (like it is done for kelp gametophytes). If this is true, the seeding of *P. palmata* is one big step closer to commercialisation.

For the first time, biomass samples of commercial cultivated *S. latissima, A. esculenta* and *L. digitata* were analysed for nearly all biochemical compounds over a sampling period of 20 months. The results add important knowledge about the biochemical composition of cultivated macroalgae in the Faroe Islands, and directly leading to support the growing macroalgae market in Faroe Islands, but also the European macroalgae community and market as such, leading to consumer awareness and datasheets for trading macroalgae.

The results confirmed (H2.1) a significant variation between the three macroalgal species of several of the biochemical compounds analysed, for example iodine. Nevertheless, many similarities between species were also found, for example that dry matter concentration goes up when ash goes down and vice versa, and that carbohydrates goes up when protein goes down and vice versa. The seasonal variation was investigated and among the analysed compounds only some varied with seasons. Our hypothesis was therefore only partly true and accepted for some of the biochemical compounds (H2.1). A product documentation considering the target compounds can thus be quantified due to the concentration found in one season, or if no seasonal variation was revealed, the overall mean can be used as documentation for the biochemical composition of the cultivated macroalgae, no matter the time of harvest. This has high value for

a company such as Ocean Rainforest to directly and well-justified guide the customers of the macroalgal product, and for the planning of future biorefinery approaches.

Surprisingly, no significant variation was revealed for the analysed compounds with respect to cultivation depths (except total carbohydrates and nitrogen for *S. latissima*) and no significant variation was found between sites (except carbohydrates for *S. latissima*). Our hypothesis was thus rejected (H2.2), but do add important information on the scale of variance for biochemical compounds of macroalgae. Commercial product documentation can thus be applied by the mean concentration of the target compounds, without distinguishing between sites or between depths of cultivation. This is true when using the investigated sites and depths described in this study but may also count in sites having similar oceanic conditions.

When structuring the knowledge about the biochemical composition and including the results of growth at sea for the kelp species a product plan was specified (**Figure 6.1**). The product plan includes a sustainability strategy that could help to ensure life below water and take climate action (see more under section 6.2 “Perspectivation”).

Overall, the cultivated species showed a promising nutritional composition as a source of food. Iodine concentrations of *S. latissima* and *L. digitata* was the only concern as alarming high concentrations was found. Contractionary, this can also be a beneficial as approximately 1/3 of the world populations has too low iodine intake. The levels of heavy metals found within this work were in similar range as other food sources and below threshold values and are therefore not concerning.

Saccharina latissima had a clean and high biomass during spring in the first, second and even third year after deployment. This biomass is well suited as food product as iodine is lowest in spring, mineral content is highest and protein concentrations are highest here. The results of high content of PUFA (47 ppm of dw) EPA and high essential amino acid score in spring (>100) make this biomass very relevant as a food result. Commercially interesting compounds was also β-carotene (10 ppm of dw) and the high content of dietary fibres (30.1 % of dw; the unknown polysaccharides) make this product interesting as a health product due to the suggested antioxidant and pro- and prebiotic properties. The autumn biomass of *S. latissima* has the highest content of glucose (7.3% of dw) and in winter the carbohydrates reach the maximal

concentration of 51.1% of dw, but is unfortunately hard to utilize and in lower quantities (**Figure 6.1**).

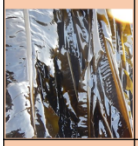
Species	Oct.	May-June 1st year	Aug-Sep 1st year	April-May 2nd year	Aug-Sep 2nd year	April-May 3rd year	Aug-Sep 3rd year	Jan. 4th year
Saccharina latissima 	Deployment of lines directly seeded	FOOD PRODUCT Low iodine High mineral High protein	FEED / EXTRACTED PRODUCTS Low ash High glucose High fucose	FOOD PRODUCT Low iodine High mineral High protein	FEED / EXTRACTED PRODUCTS Low ash High glucose High fucose	FOOD PRODUCT Low iodine High mineral High protein	FALLOWING PERIOD Habitat and feeding chamber for marine animals and birds - Ecosystem service Value: € 986/ha/yr (Buschmann et al. 2017)	BLUE-CRABON STORAGE Lines cleaned entirely and 2 T dw biomass dropped at ocean at site with water depths >200 m - 440 kg carbon, 44 kg N and 14 kg P removed Value: Unknown (discussed by Hasselström et al. 2018)
Alaria esculenta 		FOOD PRODUCT VERY LOW IODINE High mineral High protein High lipids	-	FOOD PRODUCT VERY LOW IODINE High mineral High protein High lipids	-	FOOD PRODUCT VERY LOW IODINE High mineral High protein High lipids		
Laminaria digitata 	Natural self-seeding			EXTRACTED PRODUCTS High minerals **High carbo.** High alginate High glucose High fucose	EXTRACTED PRODUCTS Low minerals **High carbo.** High alginate High glucose High fucose	EXTRACTED PRODUCTS High minerals **High carbo.** High alginate High glucose High fucose		

Figure 6.1 A harvest plan based in one Macroalgal Cultivation Rig with a surface occupation of 1 hectare and a yearly yield of 4 tonnes dw. The harvest plan includes main information on the biochemical composition of the three cultivated macroalgae *Saccharina latissima, Alaria esculenta* and *Laminaria digitata*, though "high and low" refer to the state of the species. The harvest plan also considers seeding, growth and other factors of importance like fouling and storm periods.

Alaria esculenta is a highly demanded food ingredients due to it delicious taste and it has a high crude protein concertation in spring (18.8% of dw; N*6.25). During spring *A. esculenta* excels having a 10-times lower iodine concentration than the two other species and a high EPA content (13% of all lipids), though total fatty acids is lower than *S. latissima*. However, *A. esculenta* has a lower ability to grow-out after being partial harvested, which limits it quantities. Also, the species is adapted to the wave-exposed and tidal zone and has therefore best growth in the top meters of the growth lines. Finally, this species is more exposed to grassing, and autumn harvest of this species is therefore very limited. *Alaria esculenta* is therefore an interesting by-crop as *S. latissima* is the species that is best suited for the cultivation sites in the Faroe Islands (**Figure 6.1**).

Laminaria digitata can be harvested from summer in the second year after deployment, but as a by-crop as the self-seeded individuals still only accounts for a minor part of the harvestable biomass. In the third year after deployment this species, however, has a more dominant biomass

quantity. *L. digitata* is the best source of iodine and that is no matter which season it is harvested. This species did not show specific interesting compounds and is not known as a delicious sea vegetable. The best use of this species is as in a biorefinery concept where alginate and fucoidan (3.7% fucose of dw) may be of commercial interest, nonetheless, dependent of the exact chemistry of these compounds, for example, guloronic alginate is desired (**Figure 6.1**).

The amount of collected samples (n=199) and analyses performed within this work have been comprehensive. The many variables investigated counted monthly sampling over a period of 20 months, covering three species, two sites and two water depths. All investigated factors were always sampled in triplicates to cover the natural variation. Each sample had a biomass of 1-1.5 kg ww, and after being homogenised, it represented a good exemplification. This emphasises the robustness of the results. Having said that, there is still room for many more analyses to get the full understanding of the macroalgal biomass. For example, energy (kilojoule), pigments, more detailed mono-, oligo- and polysaccharide, sodium salts, and further vitamin and mineral element analyses.

Regarding the optimisation of growth at sea, multiple partial harvesting was tested both commercially (30 km harvested) and experimentally with monthly monitoring of growth lines (n=3), and the method was found to be suitable for both *S. latissima* and *A. esculenta*, and thus confirming our hypothesis (H1.1). These multiple partial harvest results have not been tested and reported previously and thus are proven on a commercial scale for the first time. *Alaria esculenta* was harvested once a year, but over three years a total of three proved harvests without reseeding. *Saccharina latissima* was harvested two times a year with five harvests in total at the nearshore exposed site. The limiting factors of the continuous harvesting were the self-seeding from the naturally occurring species *L. digitata*, and the lifetime of the growth lines, though the latter was not investigated in this work.

By using an artificial substrate that is deployed near the sea surface at sea/open ocean, it is possible to grow macroalgae in areas that are normally too deep for the algae to grow, due to light limitations. In this work two cultivation sites and the optimal length of vertical cultivation lines in the Faroe Islands was investigated as a tool to increase yield per area. The results showed that the yield of *S. latissima* and *A. esculenta* was dependent on the cultivation site as growth

rate, average length and average yield from the commercial harvesting were higher at the nearshore exposed site than at the more sheltered site. The extended growth lines, hanging from surface and 20 m down, showed that growth was observed down to 20 m below sea level for both *S. latissima* and *A. esculenta*. The yield at the lower depths was however minimal (<100 g ww). The optimal cultivation depth was estimated from the *in situ* yield monitoring, and optimal length of the vertically hanging cultivation ropes was 10-15 m based on the ability to obtain profit. However, the 5-10 m longer growth lines had a minor impact on the aquaculture output (AO). The exact optimal length will depend on the cost of rope and the sale price of the biomass, and cannot be determined by the results of this work. The observation of *S. latissima* and *A. esculenta* being cultivated down to 20 m below sea level has not been shown in Europe previously, and is a consequence of the high light penetration in the water column in the Faroe Islands, due to for example the low concentrations of organic matter and suspended particles. Our work confirms the hypothesis that cultivation site and depth have a significant impact on the growth and yield performance can thus be confirmed, though other factors like spacing of growth lines and space between rigs had a higher significance for an increased capacity than using extended growth lines (H1.2). The calculated relative growth rates showed variations between first and second year of growth and the seasonal variation during a year, also confirming our hypothesis (H1.2). The seasonal variation of growth followed the incoming light intensities.

A MacroAlgal Cultivation Rig has proved to be suitable for cultivation in nearshore exposed sites and is likely to be useful in offshore sites as well. The AO of MACR-RUNI (~4 tonnes dw/ha/year) was low compared to traditionally longline structures used in China (~9.7 tonnes dw/ha/year). Our hypothesis (H1.3) that the MACR has a higher AO then the method used in China was therefore rejected. However, the multiple partial harvesting in up to three years may equalize the cost per tonnes macroalgae, because the Asiatic method requires new seeding every year, whereas the Faroese method can obtain biomass during three years without reseeding. These relations and assumptions need further investigation. Also, the handling space included for handling a MACR is different to the method used in China where small boats are used and spacing of horizontal longlines is being closer. Spacing of rigs has a major impact on the AO, which confirm our hypothesis (H1.3), but efficient use of space becomes less important when

the cultivation is moved away from costal areas. The yield per meter was found to be 0.29 kg dw per harvest for *S. latissima* calculated as mean of 10 m long vertical lines, which is a low yield compared to other studies, but is one of the first valid yield cultivations under open ocean conditions in a commercial scale.

Different types of MACR were tested in this work, and the MACR-RUNI has proved to be suitable for cultivation in nearshore exposed sites and is very likely suitable in offshore sites as well. The MACR-DIA was best suited for nearshore sheltered sites, not because of a higher capacity or aquaculture output, but because it is more suitable in calm seawater with lower water flow. The estimated aquaculture output for MACR-DIA was 2.25 tonnes dw per year.

Large-scale offshore cultivation still needs to be proven using the MACR, though multiple partial harvests and structural optimisation have lowered the cost noticeably. The cost of production can be further lowered from mechanization of the operation through selective breeding or value of the product can be increased by future biorefinery processes.

The main challenge for increasing the efficiency in production for a biorefinery concept of macroalgae has been to obtain a full understanding of the biochemical composition and the seasonal variation of the cultivated species. With these new results, Ocean Rainforest has unique information that can be used for detailed planning of harvest and product application.

Based on the findings of this work and including fouling patterns and storm periods, the optimal harvest seasons of *S. latissima* are in the first year of cultivation from late May to June (first harvest) and again from August to September (second harvest), and in the second and third cultivation year from April to May and again from August to September. For *A. esculenta* optimal harvest time is right before grassing initiates in mid-July, meaning that harvest must be initiated in appropriate time initial to July which is depending on scale of the operation.

In conclusion, the utilization of the ocean for large-scale macroalgal cultivation is today more likely than ever before, as until recently offshore cultivation has seemed very expensive and technically demanding. The ultimate potential in terms of valuable biomass production is so high that continued investigation in large-scale macroalgal cultivation and coherent biorefinery processes is warranted and still highly interesting.

6.2 Perspectivation

The findings of this PhD research and work suggest that macroalgae have a huge advantage compared to most arable land crops, as the macroalgal crop can be harvested several times without reseeding and produce a high yield per area. Macroalgal cultivation is a sustainable production as the algae do not need freshwater or land area, and because they can grow solely on the resources that are already plenty in oceans: light, nutrients, CO_2 and increased accessibility by seawater current.

Although current technology only delivers a small fraction of the demanded biomass, the advances in marine engineering and biotechnology indicate a significant potential for a large increase in macroalgal production. However, this will require open-ocean macroalgal cultivation, as it is the only way to increase marine biomass production without damaging already crowded and fragile coastal environments.

Conflicts between aquaculture operations, other coastal uses (e.g. traffic, infrastructure, and bird sanctuaries), and societal conflicts (e.g. tradition, "not in my backyard", visual pollution) are common and solutions must be found in careful marine spatial planning and by moving the sea cultivation more offshore. To take many sea areas into use, it would be optimal if the cultivation structures could allow normal boat trafficking to pass the cultivation fields. Today the MACR-RUNI can be passed by smaller boats with a keel less than 10 m, but this could be improved by engineering the cultivation structure.

6.2.1 SDG 12 – Responsible consumption and production

The transition to sustainable consumption and production of goods and services are necessary to reduce the negative impact on the climate and the environment and on people's health. Sustainable consumption and production patterns are therefore a prerequisite for the transition to a green economy and sustainable development.

Increased utilisation of low tropic species could help to meet these goals, as low tropic species can be produced more sustainably compared to for example salmon or beef. Macroalgae are

primary producers and thus included in the lowest trophic level. The utilization of algal biomass would have a direct positive effect on the negative impact of today's consumption.

In Asian countries, macroalgae have been consumed as marine vegetables in many years (Fleurence 2016, Makkar et al. 2016), and the main consumers have an average intake of 1.6 kg dw per year per capita (data from Japan). In European countries, the use of algae as vegetables in human nutrition has remained marginal (Fleurence 2016). Macroalgae and more particularly the brown *Laminaria hyperborea* are utilized in Europe to produce additives (e.g. alginates) or meal for animal nutrition. Thus, macroalgal stabilizing agents already play an important role in many fields of modern everyday life, either used directly or in various industrial products.

Eventhough some of the bioactive compounds of the investigated macroalgae were low (i.e. polyphenolic compounds and carotenoids), macroalgae may have an important role to improve people's health, as they are suggested to modulate chronic disease. This ability is related to unique bioactive compounds which are not present in terrestrial food sources (Brown et al. 2014). The macroalgal proteins (lectins, phycobiliproteins, peptides, and amino acids), polyphenols, and polysaccharides have also been mentioned as promising compounds with the potential to be exploited in human health applications including antiviral, anticancer and anticoagulant properties as well as having the ability to modulate gut health and risk factors for obesity and diabetes (Brown et al. 2014).

From adding macroalgae to processed foods, cardiovascular disease could be reduced, because its concentration of potassium salts does not lead to high blood pressure unlike sodium salts, that are typically used in processed food. Dried and granulated macroalgae can replace some of the flour when producing dry pasta, bread, pizza, and snack bars. In meat products, macroalgae can increase dietary fibre, retrain the moisture and could help to lower cholesterol levels (Brown et al. 2014).

Macroalgae also have umami flavour, the fifth basic taste, which is known to promote satiety and regulate food intake. The inherent health benefits in macroalgae allow them to fit naturally into the healthy snack category.

The major food and feed product attributes are thus related to natural food additives (E407-E418), flavour (umami taste), natural pigments (in feed for fish and animals), sodium reduction (major public health issue in the USA and EU), improve digestion (pre/probiotic effects), essential fatty acids (omega-3), vitamin A (β-carotene) and iodine. Other applications could be plant nutrients or chemical building blocks for many "green chemistry" applications, such as green plastics.

The perspectives of utilising macroalgae are so many that it can be difficult to distinguish which one that should be the carring element of this industry. The fact that the differences among species and sesaonal variations are so dominant require individual planing of applications considering species and time of harvest. To determne the most profitable product application other investigations are needed which was not included in this work, for example market size and possibly cascading extraction processes of valuble compounds. This is currently investigated in the EU BBI funded MACRO CASCADE project.

6.2.2 SDG 14 – Life below the water

Due to poor cultivation practices and the history of marine aquaculture operations, which to a large extend had a negative impact on the local environment, and caused harm to native species and habitats, the public perception of these impacts is often far greater than the reality. However, there are enough examples of damage and breakdowns in native systems to keep the public and decision-makers alert to potential impacts (Roesijadi et al. 2008).

Characteristics that will enhance the production of macroalgae worldwide are that the cultivation provides important ecosystem services, which need to be recognized and valued appropriately (Chopin 2014, Buck et al. 2017, Buschmann et al. 2017). If the cultivation installation is thus appropriately designed, it can serve as an engineering new installation of kelp forest and protecting emerging communities and can be used for nursery and habitat restoration. This biodiversity was indeed observed for cultivation in the Faroe Islands using the cultivation rigs (data not quantified in the present work). This could be maintained having a fallow period from summer in the third year after deployment (**Figure 6.1**). Partial harvest will also support this by leaving some “forest” at all times.

Interruption of marine mammal migration routes and feeding activities may be an issue derived from sea cultivation, and are questioned by for example NGO's, but the MACR's has a structural design that inhibits tangling of mammals. Observations at the cultivation sites in Funningsfjørður pilot showed that the structures allowed whales to pass the rigs in herds of several hundred individuals. Also, entanglement of seabirds must be avoided, and nets can be problematic compared to ropes, but the MACR has never entangled birds or other animals. Instead, the past years of work have shown a large attraction of birds that are feeding on the macroalgal or other organism biomass on the cultivation rigs, which was also discussed by (Hasselström et al. 2018).

The potential of installed macroalgal "forest" must not be neglected as the increased biodiversity will improve fish populations and potentially add fish protein for millions of people. When this ecosystem service will get a future value together with the mitigation of nutrients and carbon sequestration CO_2 the macroalgal cultivation will further increase in value.

Finally, macroalgae are known for their ability of bioremediation where nutrients are stored in the macroalgae as either reserves in the vacuole or bound into tissue structure. Nitrogen and phosphorus are the main nutrients that are taken up by algae and often the limiting nutrient for algal growth. In some areas where eutrophication is problematic macroalgae could be an important tool for remediation. One tonne dw cultivated *S. latissima* would correlate to 4 kg phosphorus and 22 kg nitrogen removed from the marine environment (**Figure 6.1**).

6.2.3 SDG 13 – Climate action

Today, excess CO_2 on our planet is problematic as the climate is getting warmer and this has a cascading effect on ecological processes of the world. Also, excess CO_2 decreases the pH of the otherwise slightly basic oceans and this is a huge problem for corals, seashells, snails and many other calcareous organisms.

By growing macroalgae, carbons are bound as organic compounds. The use of this carbon-rich biomass determines how long the storage of carbon remains before again released as CO_2. If the macroalgae are dropped at a deep-ocean location they will sink and be stored up to approximately 10,000 years, called "blue carbon storage". Opposite, if the biomass is used as

food or feeds it is stored in human or animal cell-tissue until the death of individual and thus re-cycled. In this way macroalgae are considered carbon-neutral. Sometimes the biomass can be used to replace fossil fuel products (like plastic, bioenergy etc.) or as a less heavy CO_2 emission-product than for example beef. In this way, carbon sequencing by macroalgae can be analysed using basic principles as life-cycle assessments (LCA). The input data would then be the carbon stored in the macroalgae as biomass.

Chung et al. (2011) made a critical review of CO_2 acquisition by marine macroalgae and their role as a considerable carbon sink for anthropogenic CO_2-emissions. They conclude that harvesting and appropriate use of macroalgal primary production could play a significant role in carbon sequestration and relief of greenhouse gas emissions. Chung et al. (2011) used a carbon concentration of 33% of dw macroalgal biomass, which is higher compared to the carbon concentrations of 21.7% of dw found for *S. latissima* in this study.

Chung et al. (2011) suggest using more coastlines for harvesting macroalgae and in this way remove carbon – but these coastal areas have major importance to the ocean environment as a habitat for juvenile fish, coastal protection, feeding chamber of birds and animals to mention a few of its functions. Instead of looking at the nearshore coastal areas, the idea of blue carbon sequencing should be moved further out – offshore – by providing a substrate for the algae to grow and bind carbon from – for example, the MacroAlgal Cultivation Rig.

Ramon et al. (2012) made an interesting analysis of ocean macroalgal afforestation. They estimated that if 9% of the oceans was covered with macroalgal beds then the harvested biomass could be used to produce enough biomethane to replace all of today's needs for fossil fuel energy, which today globally release 53 billion tonnes of CO_2 per year to the atmosphere (Ramon et al. 2012). By removing 53 billion tonnes CO_2 from the atmosphere and incorporate the carbon as algal tissue, 14.5 billion tonnes carbon are bound. The amount of carbon bound in *S. latissima* tissue, using the data found in this present study, would then be 66.8 billion tonnes dw *S. latissima* (~571 billion tonnes ww). Now remember that cultivated macroalgal today accounted for approximately 30 million tonnes ww globally, worth € 5 billion. The scenario by Ramon et al. (2012) with 9% of the ocean surface used for macroalgal primary production would thus increase the global production by 20000, which is not realistic.

Instead of using 9% of the Earth surface, a more realistic scenario would be the utilisation of 1% of the continental shelves for macroalgal cultivation. The continental shelves cover 5.2% of the Earth's surface and these areas have generally a water depth that is suitable for deployment of MACR's (50-200 m depth). With 1% of the continental shelfs cultivated, 306 million tonnes of CO_2 would be stored as valuable 3,300 million tonnes ww, and thus increase global production with a factor of 100.

In this scenario, the industry would be worth € 500 billion per year. The production would remove 10% of the annual CO_2 emission from Europe (total emission ~4,000 million tonnes CO_2/year). It would remediate 1.5 million tonnes phosphorus and 8.7 million tonnes nitrogen, and add artificial habitats, increase biodiversity and improve life below water. Finally, the biomass could be used as valuable food, feed and nutraceutical products, and eventually chemical building blocks. This later scenario may be realistic.

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8 Appendix

8.1 Appendix A: Sampling and analysis plan

INFORMATION ABOUT SURVEY							
Title(s):	*An investigation on the biochemical composition of selective macroalgae*						
Variables that are testet:							
	5 different species: S. latissima (SL), A. esculenta (AE) and L. digitata (LD)						
	2 different sites (A02 -moderate exposed and A71 - Exposed)						
	3 different depths (0-1, 4-5 & 9-10 meter below surface)						
	Seasonal variation samples from all year round (jan, feb, mar, …, dec)						
	Two years (2015 and 2016)						
	abbreviations						
Compounds analyzed by **DTU**:							
	AA	Amino Acid profile – Charlotte Jacobsen and co.					
	V	Vitamins (B2, alfa- and beta carotene) – Jette					
	Vd	Vitamine D – Jette					
	I	Iodine - Jens Jørgen Sloth					
	As	Arsenic – Jens Jørgen Sloth					
	Ias	Inorganic Arsenic – Jens Jørgen Sloth					
Compounds analyzed by **MATIS:**							
	LC	Total Lipid Content					
	FA	Fatty Acid Profile					
			different Fatty acids				
			C14:0	C17:0	C18:4n3		
			C15:0	C18:0	C20:1		
			C16:0	C18:1n7	C20:4		
			C16:1n9	C18:1n9	C20:5n3		
			C16:2n4	C18:2n6	C22:1n9		
			C16:3n3	C18:3n3	C22:6n3		
	n9		total n9				
	n6		total n6				
	n3		total n3				
	HM	Heavy Metals (Hg, Cd, Pb, As)					
	Hg		Mercury				
	Cd		Cadmin				
	Pb		Bly				
	As		Arsenic				
	PC	Total Protein Content					
	AO	Anti-Oxidants					
	Tpc		TPC	Total phollyphinols content			
	Orac		ORAC				
	Dpph		DPPH				
	Rp		RP				
	Mf		MF				
	CC	Total Carbohydrate Content					
	Ma		Mannitol				
	Fu		Fucoidan				
	La		Laminarin				
	Al		Alginate				

Description of analyses conducted for each sample:

NAME	DEGREE	AA	V	Vd	I	As	IAs
BLUE	Low				x	x	x
ORANGE	Middel	x			x	x	x
BROWN	High	x	x		x	x	x
PINK	Very high	x	x	x	x	x	x

Description of categories:

Blue category estimates iodine and inorganic arsenic. This is crucial to know when ORF are selling the seaweed for food perspectives. 3 replicates of each treatment. The yearly variation is described for *S. latissima* and *A. esculenta* for every month in one year, and in two depths (0-1m and 9-10m below surface) and between two sites. *P. palmata* is analyzed in Feb and April. *L. digitata* is analyzed in Feb, March and April.

Orange category includes also amino acid profile. 3 replicates of each treatment. The yearly variation is described for *S. latissima* and *A. esculenta* for every second month in one year, and in two depths (0-1m and 9-10m below surface) and between two sites for *S. latissima*. *P. palmata* is analyzed in Feb and April.

Brown category includes also Vitamins (B2+carto). 3 replicates of each treatment. The yearly variation is described for *S. latissima* and *A. esculenta* for every second month in one year. *P. palmata* is analyzed in Feb and April.

Pink category includes also Vitamin D. Two analyses, a summer and a winter sample, for *S. latissima* and *A. esculenta* is prepared and one sample of *P. palmata* from April.

Sample plan **DTU**: Numbers in **bold** is current status of samples taken

		S. latissima (SUKKERTANG)								*A. esculenta (VINGETANG)*									*Samples in to analyze*	*Total samples taken*
	Site (MACR)	A71.2			A71.0 / A71.3		A02.1			71.0 / A71	A71.2			A02.1			Wild Palmaria	Wild L. digitata		
	Depth	0-1 m	4-5 m	9-10 m	0-1 m	4-5 m	0-1 m	4-5 m	9-10 m	Wild	0-1 m	4-5 m	9-10 m	0-1 m	4-5 m	9-10 m				
	Mar-15				**3**	**3**				**1**							**1**			8
missing!	Apr-15	**3**		**2**							**3**		**2**						**8**	10
	May-15	**3**		**3**			**2**		**2**	**2**	**3**		**3**			**1**			**3**	19
	Jun-15	**3**					**3**				**3**			**2**					**11**	11
	Jul-15	**3**	**3**	**3**			**3**	**3**	**3**		**3**	**3**	**3**						**6**	27
	Aug-15	**3**					**3**				**3**								**9**	9
	Sep-15	**Ingen prøvetagning**																		0
	Oct-15	**3**	**3**	**3**							**3**	**2**	**2**						**6**	16
	Nov-15	**Ingen prøvetagning**																		0
	Dec-15	**Ingen prøvetagning**																		0
	Jan-16	**3**	**3**	**3**			**3**	**2**	**1**										**9**	15
	Feb-16	**3**					**3**				**2**						**1**	**3**	**9**	12
	Total 1st year																		61	127
	Mar-16	**3**		**3**			**3**		**3**		**3**		**3**					**3**	**12**	21
	Apr-16	**3**		**3**			**3**				**3**						**2**	**3**	**14**	17
	May-16	**3**					**3**				**3**						**1**	**3**	**13**	13
	Jun-16	**3**									**3**						1	**3**		10
	Jul-16	**3**			**3**						**3**						**1**	**3**	**12**	13
	Aug-16	**3**									**3**						1	**3**		10
	Sep-16	**3**			**3**						**3**						**1**	**3**	**12**	13
	Oct-16	3		3							3						1	3		13
	Nov-16	**3**			**3**					**3**	**3**						**3**	**3**	**12**	18
	Dec-16	3		3							3						1	3		13
	Jan-17	**3**			**3**						**3**						**1**	**3**	**12**	13
	Feb-17	3									3									6
	Total 2nd year																		87	160
	Total 2015+2016																		148	

Describtion of analyses conducted for each sample:														
previosly named		NAME	DEGREE	LC	FA	HM	PC	AO	AD	CC	Ma	Fu	La	Al
	White	Green	Low	x			x			x				
		Yellow	Middel	x		x	x			x	x	x	x	x
		Grey	High	x	x	x	x	x	x	x	x	x	x	x
	Red													

White category was used for samples in first sending, but has canged color to green (because of technical a issue)
Green category is defined by covering the seasonal variation of samples from each month in 2 years. Tests for seasonal variation, depth differences, site differences and different species being investigated. This category incluedes fa total content of fat, protein and total sugar.
Yellow category is defined by covering the seasonal variation of 5 samples per year for 2 years. It will test different sites, seasonal variations and different species. This category incluedes heavy metals (Hg, Cd, Pb, As), mannitol, laminarin, fucoidan and alginates + green category.
Grey category is defined by covering the seasonal variation of 3-4 samples per year in 2 years. It is entirely seasonal variation and different species under investigation. This category incluedes Fatty Acid Profile, Anti-oxidant, Anti-diabetic + yellow & green category.

Red category is cancelled as Matis can't provide Inorganic Arsenic, this was the only different between grey and red - all red samples are now grey.

Sample plan **MATIS**: Numbers in **bold** is current status of samples taken

	S. latissima								*A. esculenta*										
Depth	A71.2 (exposed)			A71.0 / A71.3		A02.1 (moderate exposed)			A71.0	A71.2 (exposed)			A02.1 (moderate exposed)			*P. palmata*	*L. digitata*	**Samples to analyze**	*Total sampled samples*
Site (MACR)	Top 0-1 m	Mid 4-5 m	Lower 9-10 m	Top 0-1 m	Mid 4-5 m	Top 0-1 m	Mid 4-5 m	Lower 9-10 m	Wild	Top 0-1 m	Mid 4-5 m	Lower 9-10 m	Top 0-1 m	Mid 4-5 m	Lower 9-10 m				
Mar-15				**3**	**3**				**1**							**1**		**5**	*8*
Apr-15	**Samples lost**																		*0*
May-15	**3**		**3**			**2**		**2**	**2**	**3**		**3**			**1**			**15**	*19*
Jun-15	**3**					**3**				**3**			**2**						*11*
Jul-15	**3**	**3**	**3**			**3**	**3**	**3**		**3**	**3**	**3**						**15**	*27*
Aug-15	**3**					**3**				**3**									*9*
Sep-15	**No sampling**																		*0*
Oct-15	**3**	**3**	**3**							**3**	**2**	**2**						**6**	*16*
Nov-15	**No sampling**																		*0*
Dec-15	**No sampling**																		*0*
Jan-16	**3**		**3**			**3**												**9**	*9*
Feb-16	**3**					**3**				**2**						**1**	**3**	**4**	*12*
1st year																	Total	54	*111*
Mar-16	**3**		**3**			**3**		**3**		**3**		**3**					**3**	15	*21*
Apr-16	**3**		**3**			**3**				**3**		**3**				**2**	**3**	11	*20*
May-16	**3**					**3**				**3**						1	**3**		*13*
Jun-16	**3**									**3**						1	**3**	13	*10*
Jul-16	3			3						3						1	3		*13*
Aug-16	**3**									**3**						1	**3**	13	*10*
Sep-16	3			3						3						1	3		*13*
Oct-16	**3**			**3**						**3**						1	**3**	13	*13*
Nov-16	3			3						3						1	3		*13*
Dec-16	**3**			**3**						**3**						1	**3**	13	*13*
Jan-17	3			3						3						1	3		*13*
Feb-17	**3**			**3**						**3**								9	*9*
2nd year																	Total	87	*161*
Total 2015+2016																	Total	141	*272*

8.2 Appendix B: Statistical results

Saccharina latissima		All samples			Mean				Significance of factors						
					Spring harvest	Summer harvest	Autumn harvest	Winter	Raw data					Merged data	
Compound	*Unit*	**Mean**	**SD**	**n**	**Mar-May**	**Jun-Jul**	**Aug-Oct**	**Nov-Feb**	*N.D.*	*Year*	*Month-year*	*Site*	*Depth*	*N.D.*	*Season*
Dry matter	*% of fw*	11.7	2.6	98	**10.7**	**11.5**	**12.4**	**13.2**	*trans*	no	**yes**	no	no	*yes*	**yes**
Ash	*% of dw*	38.9	6.8	113	**42.3**	**38.3**	**36.1**	**36.2**	*trans*	no	**yes**	no	no	*yes*	**yes**
Arsenic (As)	*ppm of dw*	**57.7**	19.8	54	57.9	42.3	54.8	71.7	*no*					*no*	(yes)
Inorganic arsenic (Ias)	*ppm of dw*	**0.20**	0.07	50	0.19	0.17	0.17	0.25	*no*					*no*	(yes)
Cadmium (Cd)	*ppm of dw*	**2.30**	1.10	54	2.26	2.50	2.31	2.37	*no*					*yes*	no
Mercury (Hg)	*ppm of dw*	**<0.03**	0.02	54	<0.06	<0.06	<0.06	<0.06	*no test; too low concentration*						
Lead (Pb)	*ppm of dw*	**0.20**	0.15	54	0.24	0.13	0.23	0.11	*no test; too low concentration*						
Iodine (I)	*ppm of dw*	3998	1670	65	**2825**	**3043**	**3751**	**6263**	*yes*	no	**yes**	no	no	*trans*	**yes**
Lipids	*% of dw*	**2.4**	1.3	57	2.8	2.2	2.6	1.9	*yes*	no	**yes**	no	no	*yes*	no
Σ Fatty acids	*% of dw*	**2.9**	2.0	17	3.7	-	2.6	1.9	*yes*	no	**yes**	-	-	*yes*	no
Σn3	*% of dw*	**0.7**	0.6	17	1.0	-	0.6	0.5	*yes*	no	**yes**	-	-	*no*	(no)
Σn6	*% of dw*	**0.2**	0.1	17	0.3	-	0.2	0.2	*yes*	no	no	-	-	*yes*	no
Σn9	*% of dw*	**0.4**	0.4	17	0.6	-	0.3	0.2	*yes*	no	**yes**	-	-	*yes*	no
Crude protein (N*6.25)	*% of dw*	13.0	3.0	63	**13.8**	**13.5**	**13.4**	**11.1**	*yes*	no	**yes**	no	no	*yes*	**yes**
AA-protein (ΣAA)	*% of dw*	**5.8**	1.6	35	6.6	5.1	6.0	5.3	*yes*	no	no	no	no	*yes*	no
Nitrogen	*% of dw*	2.2	0.3	63	**2.2**	**2.1**	**2.2**	**1.9**	*yes*	no	**yes**	no	**yes**	*yes*	**yes**
Carbohydrates calc.	*% of dw*	45.4	6.5	80	**41.7**	**48.0**	**45.7**	**51.1**	*yes,*year*	-	**yes**[x]	**yes**[x]	**yes**[x]	*yes*	**yes**
Total carbon (AAU)	*% of dw*	**21.7**	4.1	33	20.2	21.7	24.3	22.6	*trans*	no	no	no	-	*yes*	no
T monosaccharide (IC)	*% of dw*	14.1	9.8	27	**11.6**	-	**21.4**	**8.4**	*trans*	-	no	no	no	*yes*	**yes**
Alginate and other unknown	*% of dw*	**31.2**	-	-	30.1	-	24.3	42.7							
Mannitol	*% of dw*	**5.5**	4.0	36	4.2	5.8	9.4	2.4	*no*					*no*	(yes)
Fucose	*% of dw*	**1.9**	2.0	36	1.4	1.9	2.9	1.4	*yes*	no	no	no	-	*yes*	no
Galactose	*% of dw*	**0.6**	0.7	32	0.6	0.4	0.8	0.7	*yes*	no	**yes**	no	-	*yes*	no
Glucose	*% of dw*	4.8	3.9	32	**4.5**	**0.6**	**7.3**	**6.2**	*yes*	no	**yes**	no	-	*yes*	**yes**
Xylose/mannose	*% of dw*	**1.6**	3.0	18	2.6	-	1.2	0.2	*yes*	no	**yes**	no	-	*yes*	no
Inositol	*% of dw*	**2.1**	0.5	9	2.5	1.9	-	-	*no test; too few samples*						
Antioxidant activity															
ORAC	*µmol TE/g extract*	**14.2**	9.2	10	15.7	-	15.2	10.7	*no test; too low concentration*						
TPC	*g PGE & g GAE/100g extract*	**0.8**	0.4	13	1.1	-	0.4	0.4	*no test; too low concentration*						
Metal chelating	*mg dw/mL*	**7.8**	2.4	4	7.8	-	-	-	*no test; too low concentration*						
Reducing power	*mg dw/mL*	**5.9**	1.2	8	5.9	-	-	-	*no test; too low concentration*						
DPPH	*mg dw/mL*	**5.9**	2.5	8	5.9	-	-	-	*no test; too low concentration*						
Vitamins															
α-carotene	*ppm of dw*	n.d.			-	-	-	-	*no test; too concentration detected*						
β-carotene	*ppm of dw*	**9.49**	5.27	24	8.4	9.1	11.2	8.2	*yes*	no	no	no	-	*yes*	no
D-vitamin	*ng/g*	**<5**		2	-	<5	-	<5	*no test; too low concentration*						
Total phosphorus (AAU)	*% of dw*	**0.4**	0.2	33	0.5	0.2	0.4	0.7	*yes*	**yes**	**yes**	no	-	*no*	(yes)

Alaria esculenta

Compound	Unit	All samples Mean	SD	n	Mean: Spring harvest Mar-May	Summer harvest Jun-Jul	Autumn harvest Aug-Oct	Winter Nov-Feb	Significance of factors: Raw data N.D.	Year	month	Site	Depth	Merged data N.D.	Season
Dry matter	*% of fw*	15.8	2.4	41	**14.1**	**15.3**	**19.0**	-	*no*					*yes*	**yes**
Ash	*% of dw*	26.1	5.4	58	**28.7**	**27.2**	**22.5**	**20.7**±7.3	*trans*	yes	**yes**	no	no	*yes*	**yes**
Arsenic (As)	*ppm of dw*	**44.2**	8.4	29	43.0	45.9	43.4	47.9	*yes*	-	no	-	-	*yes*	no
Inorganic arsenic (Ias)	*ppm of dw*	**0.3**	0.1	16	0.3	0.2	0.3	0.6	*yes*	-	no	yes	-	*yes*	no
Cadmium (Cd)	*ppm of dw*	3.6	1.2	29	**4.1**	**4.4***	**2.2**	**2.7***	*yes*	-	**yes**	-	-	*yes*	**yes**
Mercury (Hg)	*ppm of dw*	**<0.03**	-	29	<0.03	<0.03	<0.03	<0.03	*no test; too low concentration*						
Lead (Pb)	*ppm of dw*	**0.3**	0.2	29	0.3	0.2	0.2	0.3	*no test; too low concentration*						
Iodine (I)	*ppm of dw*	**234.1**	119.2	30	169.4	235.1	316.5	217.0	*yes*	-	no	no	-	*yes*	no
Lipids	*% of dw*	3.1	1.0	41	**3.6**	**3.08***	**2.4**	**2.20***	*no*					*yes*	**yes**
Σ Fatty acids	*% of dw*	**2.8**	0.4	9	3.0	-	2.7	-	*no test; too few samples*						
Σn3	*% of dw*	**1.1**	0.4	9	1.3	-	1.0	-	*no test; too few samples*						
Σn6	*% of dw*	**0.2**	0.1	9	0.3	-	0.2	-	*no test; too few samples*						
Σn9	*% of dw*	**0.3**	0.1	9	0.2	-	0.3	-	*no test; too few samples*						
Crude protein (N*6.25)	*% of dw*	17.5	3.0	43	**18.8***	**16.1**	**16.5**	**17.2***	*yes*	-	**yes**	-	-	*trans*	**yes**
AA-protein (ΣAA)	*% of dw*	**8.8**	2.5	14	11.2	9.8	8.8	8.8	*yes*	-	no	-	-	*yes*	no
Nitrogen	*% of dw*	2.8	0.5	43	**3.0***	**2.6**	**2.6**	**2.8***	*yes*	-	**yes**	-	-	*trans*	**yes**
Carbohydrates calc.	*% of dw*	53.4	6.3	41	**49.8**	**53.3**	**57.5**	**59.9**	*no*					*yes*	**yes**
Total monosaccharide (IC)	*% of dw*	**16.2**	12.2	20	11.4	-	21.0	-	*trans*	-	no	-	-	*yes*	no
Alginate and other unknown	*% of dw*	**37.2**	-	-	38.4	-	36.6	-							
Mannitol	*% of dw*	**5.9**	3.9	20	4.5	5.0	7.3	-	*yes*	-	no	-	-	*yes*	no
Fucose	*% of dw*	**0.9**	0.6	20	0.7	1.3	1.1	-	*yes*	-	no	-	-	*yes*	no
Galactose	*% of dw*	**0.7**	0.6	17	1.1	0.4	0.6	-	*yes*	-	**yes**	-	-	*yes*	no
Glucose	*% of dw*	**6.6**	3.9	17	5.4	7.0	7.2	-	*yes*	-	no	-	-	*yes*	no
Xylose/mannose	*% of dw*	**1.9**	3.0	17	3.2	-	0.4	-	*yes*	-	**yes**	-	-	*no*	
Antioxidant activity															
ORAC	*µmol TE/g extract*	**63.6**	40.2	6	-	-	-	-							
TPC	*g PGE & g GAE/100g extract*	**3.8**	2.5	6	-	-	-	-							
Metal chelating	*mg dw/mL*	**0.7**	0.1	6	-	-	-	-							
Reducing power	*mg dw/mL*	**20.5**	16.2	6	-	-	-	-							
DPPH	*mg dw/mL*	**2.6**	1.0	6	-	-	-	-							
Vitamins															
α-carotene	*ppm of dw*	-		24	-	-	-	-							
β-carotene	*ppm of dw*	**22.0**	11.7	14	26.7	10.5	27.1	17.1	*yes*	-	no	-	-	*yes*	no
D-vitamin	*ng/g*	**<5**	-	1	-	-	-	-							

Laminaria digitata

Compound	Unit	All samples Mean	SD	n	Mean: Spring harvest Mar-May	Summer harvest Jun-Jul	Autumn harvest Aug-Oct	Winter Nov-Feb	Significance of factors: Raw data N.D.	Year	month	Site	Depth	Merged data N.D.	Season
Dry matter	*% of fw*	16.2	2.8	19	**12.5**	**15.0**	**19.5**	**17.7**	*yes*	-	**yes**	-	-	*yes*	**yes**
Ash	*% of dw*	32.5	5.5	16	**37.9**	**30.3**	**22.1**	**31.3**	*yes*	-	**yes**	-	-	*yes*	**yes**
Arsenic (As)	*ppm of dw*	**69.7**	28.2	6	51.0	-	-	88.4							
Inorganic arsenic (Ias)	*ppm of dw*	-	-	-	-	-	-	-							
Cadmium (Cd)	*ppm of dw*	**1.8**	0.8	6	2.4	-	-	1.1							
Mercury (Hg)	*ppm of dw*	**<0.06**	0.0	6	<0.06	-	-	<0.06							
Lead (Pb)	*ppm of dw*	**0.2**	0.1	6	0.2	-	-	0.2							
Iodine (I)	*ppm of dw*	**5361**	1449	18	4847	5509	6522	5890	*yes*	-	yes	-	-	*trans*	no
Lipids	*% of dw*	**1.5**	0.5	13	1.8	0.9	2.0	1.5	*yes*	-	no	-	-	*yes*	no
Crude protein (N*6.25)	*% of dw*	**15.6**	4.0	13	15.6	10.0	18.2	16.7	*yes*	-	yes	-	-	*yes*	no
AA-protein (ΣAA)	*% of dw*	-	-	-	-	-	-	-							
Nitrogen (DTU & MATIS)	*% of dw*	**2.5**	0.6	13	2.5	1.6	2.9	2.7	*yes*	-	yes	-	-	*yes*	no
Carbohydrates calc.	*% of dw*	**54.4**	9.5	13	45.4	58.8	57.8	46.2	*yes*	-	yes	-	-	*yes*	no
Total monosaccharide (IC)	*% of dw*	**24.4**	6.7	5	21.6	-	-	26.3							
Alginate and other unknown	*% of dw*	**30.0**	-	-	23.8	-	-	19.9							
Mannitol	*% of dw*	**10.1**	2.5	5	10.5	-	-	9.8							
Fucose	*% of dw*	**3.7**	1.1	5	4.3	-	-	3.3							
Galactose	*% of dw*	**0.8**	0.8	5	1.1	-	-	0.7							
Glucose	*% of dw*	**8.6**	6.1	5	3.4	-	-	12.1							
Xylose/mannose	*% of dw*	**1.2**	2.1	5	2.5	-	-	0.4							

8.2.1 Example of statistical results

Table 8.1 Statistical results, testing the differences between variables and their interactions for the dry matter content of *Saccharina latissima*.

Source	df	Pseudo-F	P-value	Significant
Month (season)	9	2.51	0.013	Yes
Site	1	0.46	0.496	No
Depth	1	0.09	0.759	No
Month x site	4	0.85	0.496	No
Month x depth	4	0.59	0.673	No
Site x depth	1	0.32	0.576	No
Month x site x depth	0			No test

Table 8.2 Statistical results, testing the differences between variables and their interactions for the ash content of *Saccharina latissima.*

Source	df	Pseudo-F	P-value	Significant
Month (season)	9	4.62	0.0002	Yes
Site	1	0.34	0.57	No
Depth	1	0.04	0.83	No
Month x site	7	1.12	0.35	No
Month x depth	4	0.43	0.77	No
Site x depth	0			No test
Month x site x depth	0			No test

Table 8.3 Statistical results, testing the differences between variables and their interactions for the ash content of *Alaria esculenta.*

Source	df	Pseudo-F	P-value	Significant
Year	0			No test
Month (season)	7	4.43	0.002	Yes
Depth	1	0.25	0.63	No
Year x month	2	0.30	0.73	No
Year x depth	0			No test
Month x depth	3	0.83	0.49	No
Month x site x depth	0			No test

PAPER I

Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting.

Urd Grandorf Bak, Agnes Mols-Mortensen & Ólavur Gregersen

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Production method and cost of commercial-scale offshore cultivation of kelp in the Faroe Islands using multiple partial harvesting

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ABSTRACT

The current work aimed to develop a cultivation method for macroalgae that can be applicable and economically profitable in the Atlantic Ocean. An offshore long-line macroalgal cultivation rig was designed by Ocean Rainforest Sp/f, tested in the Faroe Islands from 2010, and found suitable for cultivation in exposed and deep-water locations (water depth > 50 m). The economic risk related to lost cultivation structures was hereafter considered to be low. *Saccharina latissima* and *Alaria esculenta* were cultivated in commercial scale (5 km of growth lines). A high cost of seeding material and cost of deployment was reduced by testing multiple partial harvesting. Four non-destructive harvests were carried out in a two-year growth period without re-seeding of lines. In total, 3.2 t dry weight (dw) biomass was harvested and sold to customers within the food and cosmetic industries. The productivity was 1437.5 kg dw ha^{-1} yr^{-1} (including handling space). The 10-meter vertical growth lines had an average yield of 0.29 kg dw m^{-1} per harvest and four partial harvests were made over a 2-year period. An economic analysis showing the cost structure of important aspects of offshore macroalgae cultivation was conducted. The total cost per kg dw of cultivated *S. latissima* decreased when the number of possible harvests without re-seeding was increased (from € 36.73 to € 9.27). This work has demonstrated that large-scale kelp cultivation is possible using multiple partial harvesting in the Faroe Islands, and highlighted the need for further innovation to lower the cost per unit macroalgal produced.

1. Introduction

The need for food is increasing globally and, therefore, the efficient use of natural resources is increasingly vital. Most land areas are already utilized for the conventional agriculture of terrestrial plants. However, the oceans, that cover > 70% of the planet, potentially offer solutions for future sustainable large-scale biomass production. The use of macroalgae (seaweeds) has a long history, as does the cultivation at sea of a relatively small group of macroalgal species [1]. In North America and in Europe, macroalgae are a relatively underexploited resource, though they are the subject of an increasing interest for their potential as human food, animal feed, cosmetics, bioactive components, and biofuel [2–8]. The interest in macroalgal cultivation is driven by a market demand [1,2,8] and because of environmental concerns related to wild harvest of macroalgae [9–11].

Cultivation of macroalgae has important environmental benefits compared to harvesting wild populations. Instead of damaging natural ecosystems, new artificial marine forests are established with similar environmental functions as a nursery habitat for juvenile fish and as a food source for animals. The cultivated macroalgal biomass bioremediates nutrients and carbon (CO_2) from the surrounding environment as biomass [3,9,10]. There is therefore an ecological benefit to be gained from the cultivation of macroalgae.

The European macroalgal industry currently relies on wild harvesting, unlike Asian producers that mainly rely on cultivation. In 2015, Asia produced 27 million tonnes wet weight (ww) macroalgal biomass, corresponding to approximately 2.7 million tonnes dry weight (dw), whereas the yield in Europe was only a few hundred tonnes dw [12]. The cultivation methods developed in Asia through centuries are not easily applicable to the western countries. The reason is that the cultivation methods that are used are labour intensive, and the methods are not proven to be profitable in the Western world [2,13]. Zuniga-Jara et al. [14] made a feasibility study of offshore commercial kelp cultivation in Chile, and concluded that it was not profitable, as the sale

Abbreviations: MACR, MacroAlgae Cultivation Rig; MBSL, meter below sea level; dw, dry weight; ww, wet weight; SD, standard deviation; ha, hectare

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of the biomass was unable to cover the investment costs or the operation costs.

To reduce production cost, Burg et al. [2] described the importance of developing a cultivation system that enables multiple partial harvests. Furthermore, biofouling seems to be a major issue for cultivation in Europe [8,15–18], and the phenomenon appears to be coupled to relatively sheltered locations preventing the use of multiple partial harvesting [5,19]. Offshore cultivation, therefore, seems to be vital for a profitable macroalgal industry [5,20,21].

Offshore cultivation is defined as "the execution of activities in sites that are subject to ocean waves", which is linked to distance from shore or lack of shelter from topographical features such as islands or headlands that can mitigate the force of ocean and wind-generated waves and sites with significant wave heights of two meters or above [22].

Producing macroalgae offshore is thus promising in terms of market potential and sustainability, but an extremely challenging endeavour [2–4,7,20,23–27].

Relatively few macroalgal species have been utilized for production [1], nevertheless, kelps have been exposed to some of the first pioneer cultivation trials in North America and in Europe. The two kelp species *Saccharina latissima* (Linnaeus) Lane, Mayes, Druehl and Saunders, commonly known as "sugar kombu" or "sugar kelp", and *Alaria esculenta* (Linnaeus) Greville, with the common name "winged kelp", have attracted commercial interest for human consumption as sea-vegetables [8,17,26]. *S. latissima* grows on the lower shore in semi-exposed areas, whereas *A. esculenta* is very tolerant to more severe wave exposure. Both algae are distributed along the northern Atlantic coasts and in Arctic areas. *S. latissima* is also found along the northern Pacific coasts and is distributed in oceans with higher temperatures and lower salinities than *A. esculenta*. The cultivation techniques are well developed for both species, and especially *S. latissima* is described as having good potential for commercial-scale cultivation in Europe and in the North Atlantic [2,28].

During the past decades, several macroalgal cultivation trials have been conducted in the Atlantic Ocean in particularly using *S. latissima* [26,28–35]. However, none of these cultivation trials have resulted in large-scale profitable cultivation [2].

This paper describes the work of several years innovative large-scale kelp cultivation in the Faroe Islands, documenting the use of a new concept for offshore cultivation installation: the Macroalgal Cultivation Rig (MACR). Also, the effect on economics of multiple partial harvesting of *S. latissima* and *A. esculenta* was described for the first time. The cultivation data reflects the large variation in growth and provides a reliable base-line study of pioneer kelp cultivation for the future use in European and North American oceans.

2. Material and methods

2.1. Cultivation site and environmental conditions

The macroalgal cultivation site was located at the mouth of Funningsfjørður in the Faroe Islands (62.3030° N, 6.9267° W; Fig. 1). The Faroe Islands are an archipelago situated in the Northeast Atlantic Ocean. The site had a water depth of 50–70 m, was exposed to currents of 15–25 cm s^{-1} and was characterized as an exposed area with occasional significant wave heights of 3–6 m [36–39]. The North Atlantic Current, which originates from the warm Gulf Stream, runs past the Faroe Islands and brings warm currents to the area, providing a relatively stable seawater temperature ranging from 6 to 11 °C during the year [40]. The salinity was very stable at 35.0–35.2 [40]. Contrary to salinity, the irradiance and day length varied substantially through the year. Irradiance measured at land surface varied from < 50 μE m^{-2} s^{-1} in November to February and up to 300 μE m^{-2} s^{-1} in average during May [41]. There was a large drop in irradiance when penetrating sea surface due to reflection. In the seawater column light can penetrate down to 30–50 m below sea level (MBSL), though at these depths the irradiance was very low (< 10 μE m^{-2} s^{-1}). There was a linear relationship between the phytoplankton concentration and the attenuation coefficient, which varied between 0.05 and 0.3 m^{-1} [41].

2.2. Cultivation system

The Macroalgae Cultivation Rig (MACR) developed by the company Ocean Rainforest Sp/f was designed to withstand the conditions of the North Atlantic Ocean (Fig. 2). The MACR was constructed using lightweight and robust equipment. None of the parts were specially designed, as all equipment was bought from a local manufacturer selling fishing gear, aquaculture equipment, and equipment to the offshore industry.

The design consisted of a 500-m long polysteel fix line (30 mm in diameter) suspended horizontally at 10 MBSL (C, Fig. 2). Two main surface floats (D, Fig. 2) were connected to the fix line and 40% submerged in a static state. The mooring system consisted of four 120-m anchor lines, which were attached to the fix line and anchored to the seafloor with 1–1.5 t steel anchors (E, Fig. 2). One MACR occupied a sea surface area of 1 ha (one MACR has a nominal width of 10 m on each side of the fix line). The rig had approximately 250 growth lines (B, Fig. 2) of 10-m length attached to the fix line with a float fixed at the opposite end, stretching the lines in a vertical position.

The first test MACR was deployed in March 2010 and the growth lines attached were not seeded. After a successful structural testing period of three years, the growth lines were replaced with seeded lines with respectively *S. latissima* and *Laminaria hyperborea* (Gunnerus) Foslie. This deployment was meant as a biological test of growth. Unfortunately, these results were not consistent enough for scientific purposes, but important lessons were learnt in terms of practical handling of seeding, deployment, maintenance and harvesting. In November 2014, two more MACR's were deployed and these lines provide the data information on the growth and costs in this paper.

2.3. Seeding method

Seeding material was produced by the company Hortimare BV, located in Norway and the Netherlands, using a standard procedure for kelp sporulation [42]. Fertile *S. latissima* and *A. esculenta* were collected from wild populations in Funningsfjørður in January 2014. From the sterilized fertile sori, spore release was done by leaving the sori dehydrating and in darkness until next day. The spores were released to sterile and filtrated seawater and placed with aeration in red light at 10 ± 2 °C. The gametophytes were nursed till sufficient biomass was reached (cultivated in vitro for > 9 months). Hereafter, an induction period using white light was initiated. The gametophytes developed into juvenile sporophytes within two weeks (size < 1 mm). Density of the seeding material was approximately 0.04 mL m^{-1} seeded line or a minimum of 200 sporophytes m^{-1}. The juvenile sporophytes were seeded on 2-mm lines using a binder-mixture produced by Devan Chemicals N.V. with the product code DG518. The seed lines were twined around coils and the juvenile sporophytes were cultivated in hatchery tanks for a three-week period before deployment. The cultivation conditions as light, nutrients, waterflow, etc. is IPR of Hortimare.

2.4. Deployment

The juvenile sporophytes were deployed at sea in November 2014. The day before deployment, the seed lines (A, Fig. 2) were twined around a growth line (B, Fig. 2) of 14-mm polypropylene three strained twisted rope. The growth lines with juvenile macroalgae were stored in seawater until transportation. These growth lines summed up to a total of 470, each of 10 m length and attached along the fix line (C, Fig. 2) with a 2-m interval and each line had a buoy attached in the opposite end to provide uplift. Two MACR's were completed with 4200 m of *S. latissima* growth lines and 500 m of *A. esculenta* growth lines. The two

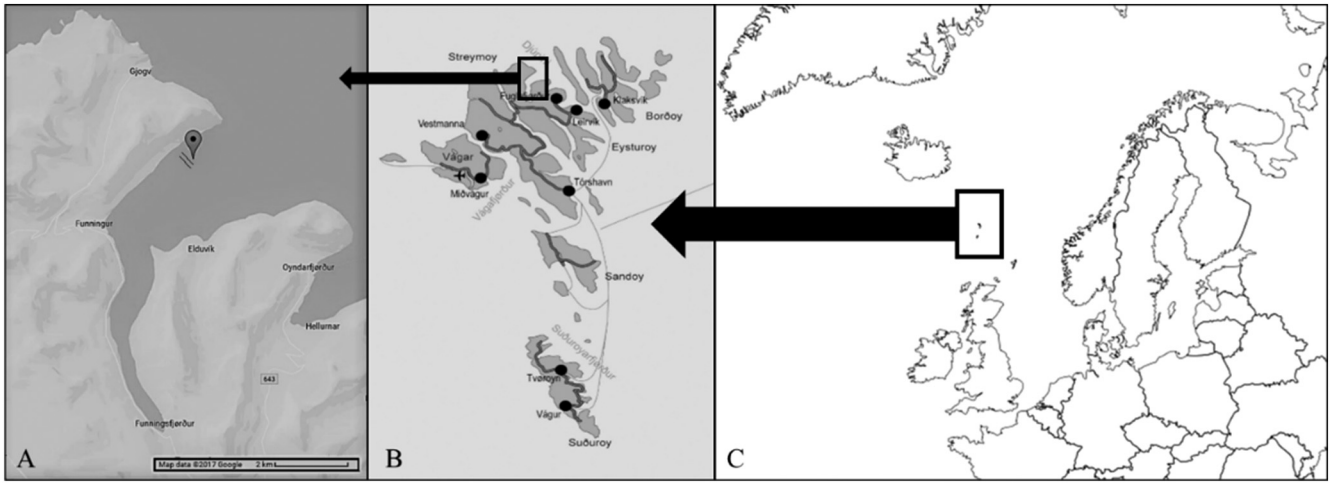

Fig. 1. Maps of the cultivation site in Funningsfjørður (A, © Google), the Faroe Islands (B, © Kort- og Martrikelstyrelsen), in the North Atlantic Ocean (C, © Wikimedia Commons, the free media repository).

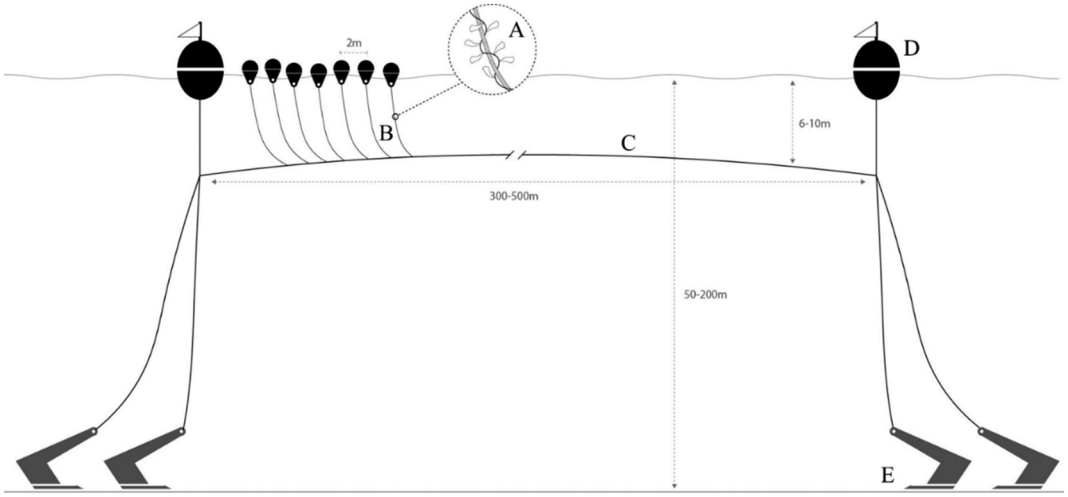

Fig. 2. Schematic drawing of a Macroalgal Cultivation Rig (MACR) constructed by Ocean Rainforest Sp/f. The construction can be deployed for macroalgal cultivation at wave-exposed sites with a water depth of 50–200 m. *Seed lines* (*A*) are twined around *growth lines* (*B*) that are attached at 2-m intervals to *the fix line* (*C*) by a loop and held in a vertical position by a buoy. Two main *surface floats* (*D*) and four *steel anchors* (*E*) ensure the right position of the rig.

rigs were named MACR1 and MACR2. All the *A. esculenta* lines were cultivated on MACR2 and the 50 lines were placed next to each other on the fix line.

2.5. Multiple partial harvesting

The biomass of *S. latissima* was harvested in early summer (27/5–29/6) and late summer (24/7–24/8) during 2015 and 2016 by the company Ocean Rainforest Sp/f. The harvest method was manual cutting with a knife. Only the blades were cut off; leaving hold-fast, stem and 5–15 cm of the blade. This cutting length was used to ensure preservation of the meristematic zone to allow re-growth (Fig. 3). The biomass of *A. esculenta* was also harvested by the non-destructive multiple partial harvesting, once in the summer 2015 and twice in the summer 2016. The optimal cutting length of *A. esculenta* has not been described previously, because the multiple partial harvesting method has not been tested for this species before. Hence, the *A. esculenta was* harvested in two different ways to find the optimal cutting length. At this farm scale level of cultivation, it was not possible to perform a randomized experimental design. Consequently, the two types of treatment were placed after each other on the fix line with 25 lines of each treatment uninterrupted. One half of the lines (n = 25) was cut proximally to the sporophylls, only leaving holdfast and part of stem, and the other half the lines (n = 25) was cut distally to the sporophylls leaving 5–15 cm of blade, entire holdfast, stem and sporophylls.

Fig. 3. Manual harvesting of *Saccharina latissima* in Funningsfjørður, Faroe Islands, August 2016.
Photo by Anja Mazuhn.

2.6. Yield, processing and storage of harvested biomass

The harvested macroalgal biomass was transported in large plastic containers (123 × 103 × 75 cm; 660 L) from the boat to the processing facilities. The biomass was cleaned carefully with seawater, before it was either dried at 35 °C for 1–2 days and packed in boxes of 80 kg dw, or immediately frozen in a chest freezer at −40 °C and stored at −20 °C in boxes of 10 kg ww.

The harvested and packed macroalgal biomass was used to describe yield. Discarded biomass was, therefore, not included in final yield calculation. Though the discarded biomass was estimated to be < 25% of total harvested biomass, this needs to be considered as an error source of yield calculations. The macroalgal biomass was occasional discarded on-board at sea, if it was covered by severe fouling, or during packaging, if colour/appearance or taste was not right. In an optimal production situation, there will be zero waste as discarded biomass will be used as feed, fertilizer, or extraction for high value molecules.

To compare the yield from this work with those described in the literature, the productivity was calculated as: *yield per unit of space per year*. The productivity of one MACR (Y_{MACR}) was the total harvested yield (Y_{Total}) from MACR1 and MACR2 divided by number of rigs (N_{rigs}) and divided by the years of harvests from the same lines (N_{years}):

$$Y_{MACR} = Y_{Total}/N_{rigs}/N_{years} = \text{kg dw/rig/yr}$$

The productivity of 1 ha was calculated using productivity of one MACR (Y_{MACR}) divided by the area occupied by one MACR (A_{MACR}):

$$AO_{MACR} = Y_{MACR}/A_{MACR} = \text{kg dw/ha/yr}$$

To calculate the sea surface area utilized in this work, it was necessary to make the following two assumptions. The area occupied by one MACR (A_{MACR}) were determined by the length of the fix line (L_{MACR}) times the width used for handling the rig (W_{MACR}) added on both sides:

Assumption 1.

$$A_{MACR} = L_{MACR} \cdot 2(W_{MACR}), \text{ in this case: } 500\ m \cdot 2\ (10\ m) = 10{,}000\ m^2 = 1\ ha.$$

Thus, one MACR occupied a sea surface area of one hectare (ha) including space for handling the rig, as the fix lines used was 500-m, and the width used for handling was set to 10-m of space added to both sides of the fix line.

The area occupied by one vertical growth line (A_{GL}) was determined by the length between growth lines (Lb_{GL}) times the width which a growth line occupies (W_{GL}):

Assumption 2.

$$A_{GL} = Lb_{GL} \cdot W_{GL}, \text{ in this case: } 2\ m \cdot 0.5\ m = 1\ m^2.$$

Thus, one growth line occupied a sea surface area of one square meter (m^2) not including space for handling the line, as the length between growth lines was two meters, and the width was set to be a half meter based on the occupied space of a buoy.

2.7. Conversion factor between fresh and dry macroalgae biomass

In all calculations of wet weight (ww) biomass to dried biomass (dw) or opposite a conversion-factor of 10:1 was used [43,44]. All cultivation results described as yield in dw, and reference to results from the literature was also described as dw, but also as ww, if they were initially recorded or presented in ww.

2.8. Growth measurement

Growth of *S. latissima* deployed in November 2014 was monitored monthly over a period of two years. For each meter the six longest algal individuals were measured as technical replicates. The average of each meter was used to describe the average maximal length and compared with biological replicates (n = 3). The length was measured from growth line to tip of alga. The length was measured at five different depth intervals (1–2, 3–4, 5–6, 7–8 and 9–10 MBSL) and the average length of the vertical growth line was used (n = 3). Note that the surface meter was not used, though this was often the best producing meter, but here other species often overtook the line e.g. *A. esculenta* and *Laminaria digitata* (Hudson) J.V. Lamouroux. The average maximal length was used for statistical treatment to compare replicate lines, cultivation in depth, and in different seasons.

Also, the number of visible individuals on each meter was counted and used in the data treatment. Number of individuals and average maximal length provided a comparable dataset for growth under various conditions, but was not used to calculate the yield.

Finally, a field inspection was made to determine length and yield per meter line and at different depths prior to harvesting. The average maximal length of the macroalgae on the inspection days was the same data as for the seasonal variation in growth. For the yield calculations, a meter of line was harvested and weighted on board using a hand-held scale (n = 2–4). The field inspections were made May 2015, Aug 2015, and May 2016 at ten different depths (0–1, 1–2, … 9–10 MBSL). These numbers of yield serve as a yield-calculation without errors of discarded biomass.

The study did not include a control treatment: non-harvested lines growing throughout 2014–2016. Data of growth and yield could, therefore, not be compared to non-harvested reference lines.

2.9. Formulation of cost functions

To show the underlying cost structure of macroalgal cultivation using a MACR we formulated the cost functions. The yearly cost of investment in terms of capital expenditure (CAPEX) on a MACR was subdivided into expenditure on the cultivation rig (TC_{RIG}) and on the growth lines (TC_{GL}), divided by the number of years over which the rig (d_{RIG}) and the growth lines (d_{GL}) were depreciated:

$$CAPEX_{TOTAL} = TC_{RIG}/d_{RIG} + TC_{GL}/d_{GL}$$

where TC_{RIG} was the total cost of the rig and TC_{GL} was the total cost of the growth lines. The total cost of a rig was subdivided into the sum of material costs (MC_{RIG}) and deployment costs (DC_{RIG}):

$$TC_{RIG} = MC_{RIG} + DC_{RIG}$$

The material costs (MC_{RIG}) included costs of anchors, chains, the fix line, fittings, surface floats and signal buoys. The deployment costs (DC) were the hourly cost of vessel operation offshore, the hourly cost of labour and the total number of operation hours needed for deployment. To determine the total cost of the growth lines (TC_{GL}) on a MACR-installation, the cost of a single growth line (C_{GL}) was multiplied by the number of growth lines (N_{GL}):

$$TC_{GL} = N_{GL} \cdot C_{GL}$$

The deployment and installation of growth lines included material for preparation, strips, and tape, twining seed lines around growth lines and transport of growth lines for installation offshore on the fix line.

The operational expenditures (OPEX) involved cultivation offshore, monitoring, maintenance, and harvesting, and were formulated as follows:

$$OPEX = OC \cdot N_{\text{harvests per year}}$$

where $N_{\text{harvests per year}}$ indicates the number of harvests in a year and OC is the operational cost for one growth period. The operational cost (OC)

was the hourly cost of vessel operation offshore, plus the hourly cost of labour, multiplied by the number of hours spent on each inspection times the number of inspections in each growth period, plus the time spent on harvesting each growth line times by the number of growth lines.

$$OC = (p_{vessel} + p_{labour})\cdot(q_{inspection\ hours}\cdot N_{inspections} + q_{harvesting\ hours}\cdot N_{GL})$$

To determine the revenue function and the cost per unit macroalgae we identified the yield of harvested biomass during the growth period. The yield (Y) in kg dw is calculated as:

$$Y = G\cdot N_{GL}\cdot q_{rope}\cdot N_{harvests\ per\ year}$$

where G is the average growth of macroalgae per growth period in kg dw per meter growth line, N_{GL} is the number of growth lines, and q_{rope} is the length of each growth line. The average cost per unit algal biomass (AVC) was calculated as:

$$AVC = (CAPEX + OPEX)/Y$$

3. Results

3.1. Cultivation system

Since deployment of the first MACR in March 2010 and the following two MACR's deployed in 2014, the installations have remained intact in the ocean and have proven themselves able to withstand the physical strains caused by the energetic wave climate and storms of the North Atlantic Ocean.

3.2. Harvest and yield

The two 500-m long MACR's that were deployed at the cultivation site in November 2014 had a total of 470 vertical 10-m long growth lines. The total of growth lines seeded with *Saccharina latissima* was 420 and for *Alaria esculenta* 50 lines. The *S. latissima* lines were partially harvested four times and the *A. esculenta* lines were partially harvested three times within the harvest season in 2015 and 2016 (Table 1). The total biomass harvested (Y_{Total}) over two years for all three seaweed species was 3517.6 kg dw. The annual productivity of one MACR (Y_{MACR}) was thus 879.4 kg dw yr^{-1}.

On average, one MACR was harvested within 6.25 working days. Mean handling time per line ± SD was 10 ± 5 min from the first three harvests not including the time used to arrive at the location. The time used for sailing was not included thus comparison between studies is possible even though the cultivation sites are placed at different distance from a harbour.

After introducing modifications on the deck of the harvesting vessel the handling time was reduced to 8 ± 1 min in average per line ± SD (Table 1). These modifications consisted of a frame that was made from 2-m long PVC pipes, 15 cm in diameter, standing vertically in a square on the vessel. Between each of the vertical pipes similar horizontal pipes were attached. When harvesting a line, the vessel crane pulled up a line and placed it on top of the frame. Hereafter, the macroalgae was harvested using gravity into storage plastic containers on board. The new harvest method using a frame reduced average working days on one MACR to 3.5 days of harvesting per MACR compared to the 6.25 days (Table 1). The macroalgae biomass was stored in containers on board and in average 2.6 ± 1.1 (mean ± SD) line would fill one container.

Harvested yield was registered (weighted) after either drying or freezing. This meant that discarded biomass during handling were not registered. Also, the yield left on lines for re-growth was not monitored or used in yield calculation. This must be kept in mind when comparing yield between studies. For each harvest, not all growth lines were cut (Table 1). The excluded lines were either affected by entanglement, had too low yield or were used for other purposes than sale e.g. research.

Table 1
Results of multiple partial harvesting from two Macroalgae Cultivation Rig's (MACR) deployed offshore in the Faroe Islands. MACR 1 had 240 and MACR 2 had 230 of 10-m long growth lines attached. Results marked with * were estimated from total harvest of 2015 using number of lines harvested in each period. Results marked with # were possibly overestimated because self-seeded *Alaria esculenta* growing on *Saccharina latissima*-lines were included in the yield calculation.

Deployed: Nov. 2014	MACR	1st harvest	2nd harvest	3rd harvest	4th harvest
Harvest periods	1	May/ June 2015	July/Aug. 2015	May/ June 2016	July/Aug. 2016
	2	June 2015	Aug./ Sept. 2015	May/ June 2016	Aug. 2016
Days used	1	8	9	7	4
	2	4	6	9	3
Meters harvested	1	610	1700	1600	1400
	2	830	1250	1980	1330
% harvested of total deployed	1	25%	71%	67%	58%
	2	36%	54%	86%	58%
Yield *S. latissima* in total	kg dw	347.2	787.8	1026.3	527.4
	Meter line	*1190	*2700	3080	2230
	kg dw m^{-1}	*0.292	*0.292	0.333	0.237
Yield *A. esculenta* in total	kg dw	0	416.8	165.4	28.8
	Meter line		#500	#250	#250
	kg dw m^{-1}		0.834	0.662	0.115
Yield *L. digitata* in total	kg dw	0	0	113.3	104.6
Total harvested yield (Y_{Total})	kg dw				3517.0

All the biomass was sold mainly as dried for the food market, and a small quantity as frozen to the cosmetic industry. The sale strategy is based on a "business to business" market implicating that the company Ocean Rainforest Sp/f does not carry its own retail brand.

3.2.1. Saccharina latissima

The multiple partial harvesting method was used when *S. latissima* was harvested and the yield increased at each harvest from the first to the third (Fig. 4). Though, the fourth harvest in July/August 2016 had a lower total yield than the previous. The total harvested biomass of *S.*

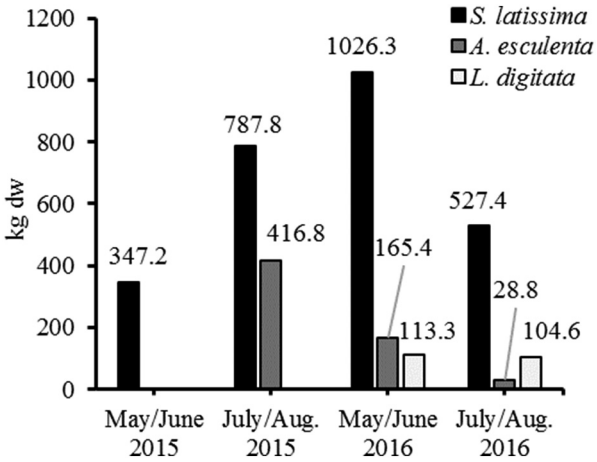

Fig. 4. Total yield from 4200-m *Saccharina latissima* and 500-m *Alaria esculenta*-lines in kg dry weight. The biomass was deployed offshore in the Faroe Islands in November 2014, and multiple partial harvested four times without re-seeding of growth lines. In 2016, self-seeded *Laminaria digitata* had overtaken some lines or part of lines. All three species were harvested, dried or frozen and sold to the food and cosmetic market. The yields represent biomass ready for sale; discarded biomass and biomass left for re-growth were not included.

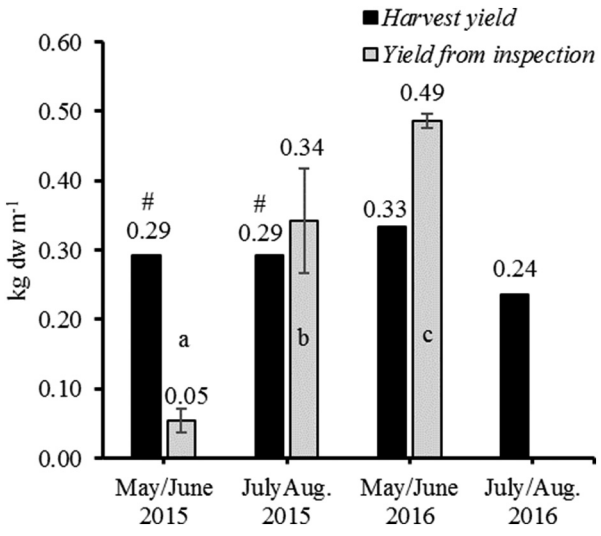

Fig. 5. Average yield of *Saccharina latissima* in dry weight of one-meter growth line. The biomass was deployed offshore in the Faroe Islands in November 2014, and multiple partial harvested four times without re-seeding of growth lines. Yield per meter was determined either by harvested biomass divided by meter of harvested line (black) or by field inspections (n = 5), error bars represent standard deviation. Statistical different harvest yields are marked with different letters. Pillars marked with # was calculated from the total 2015-yield and not for each harvest. The yield represents biomass ready for sale and discarded biomass and biomass left for re-growth were not included.

latissima from two years was 2688.7 kg dw. This yield represented harvest of 9200 m of growth line distributed over four harvests of two MACR's (Table 1). The variation in yield between the different growth lines was large (0.35–7.66 kg dw per line). The average yield per meter of line was determined by field inspections and from total yield per harvest divided by meter of harvested lines. The average yield per meter determined from inspections showed a significant increase between each harvest (One-way ANOVA, P = 0.0004, n = 5; Fig. 5). The average yield per meter also increased between the first harvests looking at the harvest yield data (Fig. 5), but it decreased for the fourth one. The macroalgae harvested during 2015 was registered only from the year of harvest and not specified to the harvest turns. Accordingly, the yield of 2015 was divided between the two harvest turns by the number of lines harvested in each turn (1st or 2nd harvest). This number was known due to a registration system used on-board of the vessel of harvested lines (Fig. 4). The average yield per meter in 2015 was accordingly calculated from a total amount harvested biomass divided by total lines harvested in 2015. To improve the yield per meter calculation, the yield was also measured from field inspections before each harvest turn. The yield found from inspections, was hence more accurately describing the yield per meter growth line (Fig. 5).

3.2.2. *Alaria esculenta*

Re-growth from multiple partial harvest of cultivated *A. esculenta* was not previously tested. After deployment in November 2014, *A. esculenta* was partially harvested the first time in summer 2015. In early spring 2016 re-growth was observed. The re-growth proved that multiple partial harvest of this species is possible. The algae were cut in two different ways (see method and material Section 2.4) and only lines cut distally of the sporophylls showed re-growth.

First harvest of *A. esculenta* in July 2015 had a total yield of 417 kg dw from 500-m growth line (Fig. 4). This gives an average yield per meter growth line of 0.834 kg dw per meter. This yield per meter is not a valid estimation, because self-seeded *A. esculenta* on *S. latissima* lines were also harvested and counted together with yield from seeded *A. esculenta* lines. An *A. esculenta* seeded line, had approximately a yield of 1–1.5 kg dw with the largest part growing on the first two MBSL (no data shown). For 50 lines, this would result in a yield between 50 and 75 kg dw. Hence, the harvested yield of 417 kg dw was more than five times the yield one could expect. Consequently, we cannot describe the yield per meter growth line or conduct cost calculations for this species.

The second harvest of *A. esculenta* in May/June 2016 had a yield of 165 kg dw (Fig. 4), meaning an average yield of 0.33 kg dw per meter calculated from 500-m of growth line. This second harvest was crucial for proof of concept of multiple partial harvesting of *A. esculenta*, but not in terms of yield as half of lines were wrongly cut (proximate to the sporophylls) and had, therefore, no biomass. Consequently, a corrected calculation of average yield per meter of growth line was 0.66 kg dw per meter using harvest from 250-m of growth line. *A. esculenta* harvested from *S. latissima* growth lines was continuously used for sale and the yield of *A. esculenta* from these lines feeds into the total harvested yield and makes the calculation of yield per meter even more invalid. The wrongly cut lines were overtaken by self-seeded *L. digitata*.

The *A. esculenta* lines showed promising yield for the third harvest in early July 2016, but the biomass had deteriorated significantly one month later when the harvest was planned to take place. Only 28.8 kg dw were harvested in August 2016 (Fig. 4). This observation advises to conduct the harvesting by mid-July at the latest.

3.3. Seasonal variation and variation with depth of *Saccharina latissima*

Saccharina latissima was harvested when maximal average length was 116.2 ± 9.4 (mean ± SD) cm and the longest *S. latissima* individual was measured to 329 cm. The highest growth was seen during summer (April–September) and degradation or low growth was seen

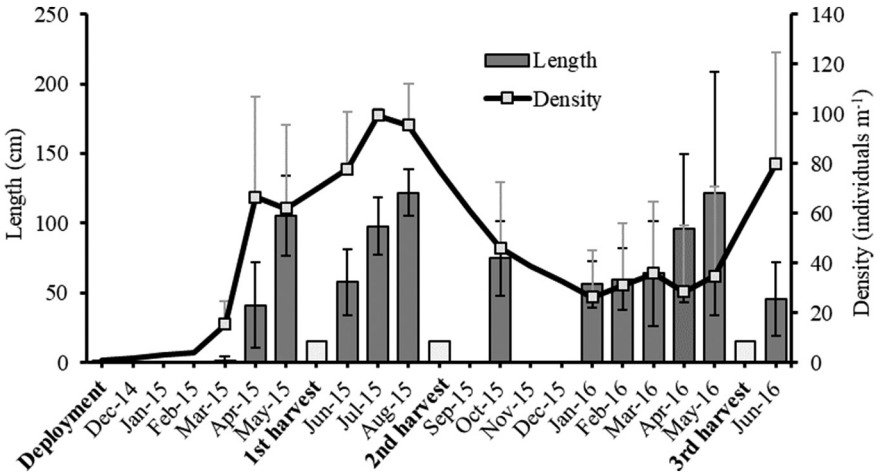

Fig. 6. Growth (average maximal length in cm ± SD, black lines) and density (average number of visible individuals per meter + SD, grey lines) of *Saccharina latissima* (n = 15). The biomass was deployed on 10-m long vertical growth lines offshore in the Faroe Islands in November 2014, monitored monthly, and harvested three times by multiple partial harvesting. The blades were cut 5–15 cm above growth line leaving holdfast, stem and part of blade with meristematic tissue. The length after harvest is illustrated as lighter pillars, though not measured.

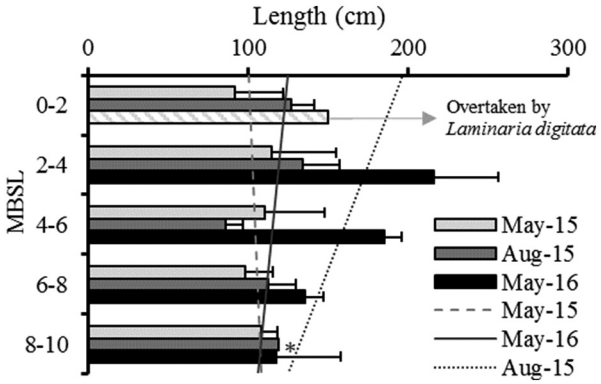

Fig. 7. Mean length in cm of cultivated *Saccharina latissima* (n = 3), error bars represent standard deviation. The biomass was deployed on 10-m long vertical growth lines offshore in the Faroe Islands in November 2014, monitored before harvest in May and August 2015 and in May 2016. The length was measured in five different depth intervals 0–2, 2–4, 4–6, 6–8, and 8–10 m below sea level (MBSL). For each season, a linear trend line was used to show growth trend with decreasing water depths. Pillar marked with * had one replicate. In the second year of cultivation the top meters (0–2 MBSL) were overtaken by *Laminaria digitata*.

during winter (November–February; Fig. 6). The seasonal growth pattern was interrupted by two harvests each year during summer months. A growth pattern not disturbed by harvesting was not monitored.

Each meter of growth line had a mean of 64.1 ± 30.3 (mean ± SD) visible individuals. The large variation in density (Fig. 6) describes an uneven distribution of individuals on the growth lines. This gives room for optimised seeding techniques. There was a large decrease in density after second harvest, which could be due to the multiple partial harvest method. After third harvest, the density was again increasing to 80 individuals per meter. The explanation of this later increase might be self-seeding from wild populations.

The growth in depth by length is illustrate in Fig. 7. To investigate the maximal cultivation depth below sea level a linear trend line was determined using the monitored growth of *S. latissima* at five depth intervals: 0–2, 2–4, 4–6, 6–8 and 8–10 MBSL (Table 2). The linear regression line of the length-measurements showed that *S. latissima* in May/June 2015 had an increased length with increased depths (slope 1.65 cm) and a mean length of the macroalgae of 99.60 cm at surface level (0 MBSL), probably because no shading occurs when crop was still young (age < 6 month), and in this way not light limited. The July/August 2015 harvest had a slight decrease in growth (slope −3.79 cm) with decreasing depths and a mean surface length of 125.6 cm. The May/June 2016 harvest had a stronger decrease with depths (slope − 14.48 cm) and a larger mean surface length of 208.9 cm. The steeper slope found in May/June 2016 indicates that shading from the proximally algae is more significant for older lines. Hence, the growth during July/August 2015 and May/June 2016 showed light limitation with increased depths.

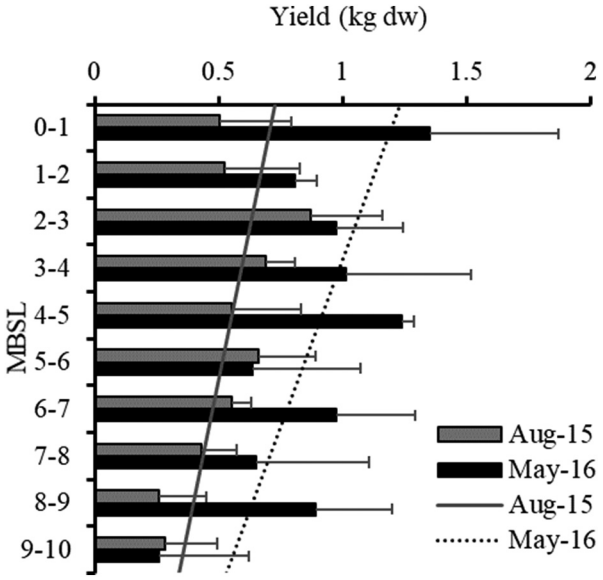

Fig. 8. Measured mean yield in kg dry weight (dw) per meter of *Saccharina latissima* growth line deployed in November 2014 offshore in the Faroe Islands (Aug-15 n = 4, May-16 n = 2), error bars represent standard deviation. The yield was monitored during field inspections before harvest in August 2015 and May 2016. The lines were harvested four times without re-seeding of growth lines (no data for yield before harvest in May/June 2015 and July/August 2016). Yield was found for each meter from sea surface to 10 m below sea level (MBSL). For each season, a linear trend line was used to show growth trend with decreasing water depths.

A similar pattern of slightly decreasing yield was seen from field inspection of yield (kg dw) at different depths (Fig. 8). Before harvest in July/August 2015 (2nd harvest) and May/June 2016 (3rd harvest), the yield from each meter in general decreased with increased depth below surface, though some depth-meters had higher yield than the meter above. This pattern could generate from few data points, competition between species, or human disturbance (e.g. tangling of lines).

The seasonal variation and the variation in growth with depths were normally distributed (Shapiro-Wilk normality test, alpha = 0.05) and the data was, therefore, compared by a parametrical Tukey *t*-test (Two-way ANOVA, unpaired, alpha = 0.05; Table 3). The interaction of depth and seasons was found significant ($P < 0.0001$), indication that seasonal variation (light variation) influences the growth in depth. During summer when it is brighter the macroalgae can grow at deeper water depths than during winter were light is limited.

The take-home message is that yield decreases with depth as light decreases through the effects of attenuation and shading and the growth season in the Faroe Islands is from April to October where light intensity is highest.

Table 2
Linear trend lines for cultivated *Saccharina latissima* deployed offshore in the Faroe Islands in November 2014 using 10-m long vertical growth lines on a Macroalgae Cultivation Rig (MACR). Data for both length and yield measurements was used (Figs. 7 and 8). Mean length/yield at water surface (x = 0) and maximal cultivation depth in meters below sea level (MBSL) were estimated, corresponding to no growth/no biomass (y = 0).

Saccharina latissima		May/June 2015	July/Aug. 2015	May/June 2016	July/Aug. 2016
Length	Linear trend line	y = 1.65x + 99.60	y = −3.79x + 127.17	y = −14.48x + 204.46	N/A
	Mean length at surface (cm)	99.60	127.17	204.46	
	Max. cultivation depth (MBSL)		33.55	14.12	
	R^2	0.0776	0.1053	0.3348	
Yield	Linear trend line	N/A	y = −0.0384x + 0.7444	y = −0.07x + 1.2655	N/A
	Mean yield at surface (kg dw)		0.7444	1.2655	
	Max. cultivation depth (MBSL)		19.39	18.08	
	R^2		0.3988	0.4577	

Table 3
Statistical output of an Ordinary two-way ANOVA, unpaired, Tukey test in GraphPad Prism 7.02. Alpha = 0.05.

Source of variation	% of total variation	P value	Significant
Interaction (depth × month) (df 36)	22.93	< 0.0001	Yes
Months (df 12)	39.43	< 0.0001	Yes
Depth (df 3)	1.93	0.0258	Yes

3.4. Analysis of cost and important scale aspects

The cost per kg cultivated *S. latissima* was determined from capital expenditure (CAPEX), operational expenditure (OPEX) and yield (Y) (Table 4). The cost of *A. esculenta* was not conducted because of 1) unrealistic average yield per meter of growth line, 2) half of the lines were harvested by a wrong method, and 3) a significant deterioration of biomass was seen during July/August 2016.

The cost calculations for *S. latissima* were based on the following assumptions: One MACR has a 500-m horizontal fix line with 250 growth lines attached to it, and each growth line is 10-m long, so that the total growth capacity is 2500-m of growth lines. The harvesting yield was based on the cultivation from two MACR seeded with *S. latissima* in November 2014 and harvested during 2015 and 2016. The result of this large-scale cultivation showed that *S. latissima* was suitable for harvest twice a year.

Based on findings within this survey, the average total cost (TC_{RIG}) of installing one MACR was € 34,000, and the average total cost (TC_{GL}) of installing the growth lines on one MACR was € 14,900. The operational cost (OC) for one MACR was € 4700 per growth period. Besides the cost of rig and growth lines, CAPEX depended on their durability. The result of cultivating kelp offshore showed that a rig was worn after 5 years at sea ($d_{RIG} = 5$), and the growth lines were depreciated after 3 years ($d_{GL} = 3$).

The base scenario was a single year and one harvest with 0.29 kg dw *S. latissima* per meter. In the base scenario, the growth lines were replaced every year. From the cost per kg dw *S. latissima*, it was seen that the CAPEX was 82% and OPEX was 18% of the total cost per kg biomass dw. In the base scenario, cultivation of 1 kg *S. latissima* dw was € 36.73 (Fig. 9).

The total cost per kg dw cultivated biomass decreased from multiple harvesting (alternative scenarios) and the total cost per kg dw *S. latissima* ended at € 9.27. The total cost of rig and growth lines per kg dw biomass were declining due to the increase in yield from multiple harvests. However, the operational cost that relied on number of harvests per year and yield remained stable.

The cost per kg macroalgae was very much dependent on the total number of harvests from growth lines without re-seeding and the yield per meter. In addition, the total cost indicates more room for cost reduction in operational costs compared to the cost of capital expenditures (CAPEX).

4. Discussion

4.1. Survivability of the macroalgal cultivation rig

The test of the MACR has proved that deployment of installation and growth lines, monitoring, and harvest is possible and easy to handle even though the test site was exposed to waves and placed at a location with a water depth of > 50 m. Its durability has been successfully determined to five years and the structures tested have survived the physical stress at the cultivation site since 2010. Based on these results, we consider the economic risk related to lost cultivation structures to be low.

The properties of a MACR for large-scale offshore macroalgal cultivation are e.g. the easy handling of the vertical growth lines that enables the production to switch from one species to another between harvests, thus optimising year-round utilisation and commercial performance. The detach of growth lines will, however, be somewhat challenging due to fouling growing on the knots. This can be prevented, if lines are handled frequently and, in this way, avoiding establishment of severe fouling.

Another advantage of the rig was the flexible structure of the growth lines, as they bend down to a horizontal position under severe weather conditions moving out of the damaging near sea surface zone. The flexibility was also important when boats were sailing through the cultivation area and the lines could bend or move away from the boat without being damaged.

Burg et al. [2] described the importance of a system that enables

Table 4
Cost calculations for cultivated kelp at an offshore and exposed site in the Faroe Islands using the special designed Macroalgae Cultivation Rig (MACR). This case represents one MACR seeded with 2500 m of *Saccharina latissima*.

Case: One MACR with *Saccharina latissima*	Equation	Base scenario	Alternative scenarios		
Production data					
Total meters of growth line	$N_{GL} \cdot q_{rope}$	2500	2500	2500	2500
Years with same growth lines		1	1	2	3
No. of harvests per year	$N_{harvests}$	1	2	2	2
Total no. of harvests without re-seeding		1	2	4	6
Average yield per meter growth line per harvest (kg dw)	G	0.29	0.32	0.37	0.29
Total cumulated yield per meter growth line (kg dw)		0.29	0.58	1.15	1.73
Total yield per growth line/m^2 sea surface (kg dw)		2.88	5.75	11.50	17.25
Total yield per MACR/1 ha (kg dw)		718.75	1437.50	2875.00	4312.50
Annual yield of harvested biomass (kg dw)	Y	718.75	1437.50	1437.50	1437.50
Economic data					
Cost of rig per year	TC_{RIG}/d_{RIG}	€ 6800	€ 6800	€ 6800	€ 6800
Cost of growth lines per year (2500 m)	TC_{GL}/d_{GL}	€ 14,900	€ 14,900	€ 7450	€ 4967
Capital expenditure per year	CAPEX	€ 21,700	€ 21,700	€ 14,250	€ 11,767
Operating cost per year	OPEX	€ 4700	€ 9400	€ 9400	€ 9400
Total cost per year		€ 26,400	€ 31,100	€ 23,650	€ 21,167
Total cumulated cost per MACR		€ 26,400	€ 31,100	€ 47,300	€ 63,500
Costs					
Cost of rig per kg macroalgae (dw)		€ 9.46	€ 4.73	€ 2.37	€ 1.58
Cost of growth lines per kg macroalgae (dw)		€ 20.73	€ 10.37	€ 2.59	€ 1.15
Operating cost per kg macroalgae (dw)		€ 6.54	€ 6.54	€ 6.54	€ 6.54
Total cost per kg macroalgae (dw)		€ 36.73	€ 21.63	€ 11.50	€ 9.27

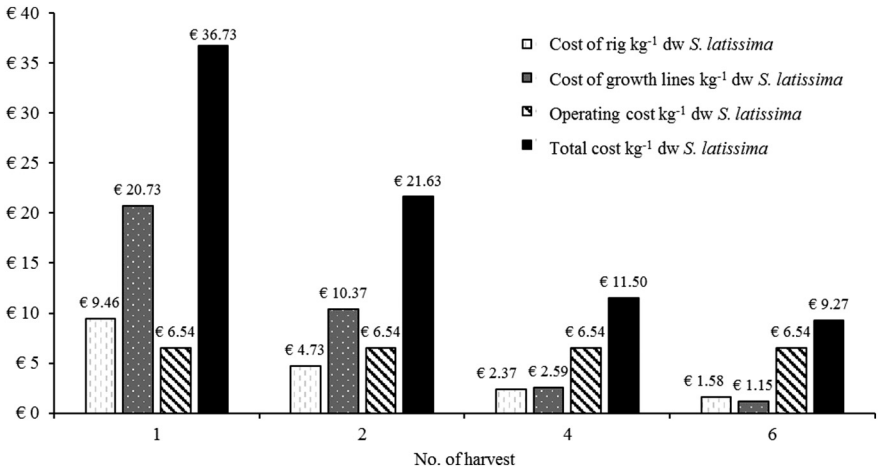

Fig. 9. Cost distribution for different number of harvests from the same growth lines with one (base scenario), two, four or six harvests without re-seeding. The calculation was based on data from two years of *Saccharina latissima* cultivation offshore in the Faroe Islands using two Macroalgae Cultivation Rig (MACR), a total of 5 km growth line.

multiple harvests to reduce production costs, and this was successfully proven by the current work with four partial harvests within 16-months. The approach of this work has demonstrated the potential for large-scale macroalgal production at deep-water locations in the North Atlantic region.

4.2. Multiple partial harvesting

A common harvest method for Laminarian species has been to cut the entire blade right at the end of the fast-growing period lasting from January to May in Europe [5,27]. This method requires re-seeding following every harvest, and will, thus, result in higher cost compared to multiple partial harvesting. Several European studies have shown that harvest later than April/May resulted in high fouling, which made the application for food impossible [5,8,15–18]. The phenomenon of fouling appears to be coupled to relatively sheltered locations [5]. In contrast to other locations in Europe, the fast growing-period in the Faroe Islands was from March to October, and less fouling occurred as seawater temperature remained low (< 11 °C in summer). Instead of harvesting entire blades followed by re-seeding, this study presented a harvest method that partially cut the blade several times within the life span of the alga lowering the cost per unit macroalgae. Our outcome was most likely related to both physical conditions of the Faroe Islands and the harsh conditions of offshore cultivation: deep-sea location, strong current, and high wave actions (Section 2.1).

Our results showed that at least four harvests were possible without re-seeding *S. latissima* and three harvests for *A. esculenta* within a period of 16 months, when cut distally to the sporophylls. This has not been proven until now and has major impact on the cost related to seeding, growth lines, and deployment.

The most common harvest method, where the entire blade is cut off, was also used for *Saccharina japonica* in northern China after a two-years growth period. Zhang et al. [27] presented their average blade length of second-year algae to be 2.7 m, having an average yield per alga of 0.14 kg dw (1.4 kg ww), and a density of 14–16 individuals per meter of horizontal longline. In our study, *S. latissima* were harvested when the average maximal length was 116.2 cm and the sporophyte density was 64 individuals per meter of line (average of all harvests, Fig. 6). The average yield from all depths and from all four harvests was 0.29 kg dw per meter (total harvested biomass, Fig. 5). Initially, the method used in the northern China seems to generate a higher yield per meter. On the other hand, did our cultivation have the advantage of four harvests without re-seeding, and the use of vertical cultivation lines, thus expanding the cultivation from 2D to 3D. The question is therefore if higher density, less biomass, 3D cultivation, and a multiple partial harvesting is preferable instead of one harvest of the entire alga in the second year of cultivation?

Zhang et al. [27] had a yield per meter horizontal growth line of 2.21 kg dw (= 0.138 kg dw per alga * 16 individuals per meter), corresponding to yield per m^2 sea surface not including handling space. In our case, using multiple partial harvesting, the yield per m^2 sea surface was 11.5 kg dw (Table 4). Almost five times more than Zhang et al. [27].

Opposite numbers were seen when including handling space as Zhang et al. [27] used 100-meter-long horizontal lines with four meters in between the lines. This was equal to 25 lines in 1 ha given in total 2500-m growth line in 1 ha. The total yield per ha including handling space was therefore 5527.0 kg dw (= 2.21 kg dw * 2500 m line). In our case with *S. latissima*, the yield per ha from four harvests including handling space was 2875 kg dw (Table 4). Only half the yield of Zhang et al. [27].

This emphasises, that yield per area is very depended on the way it is estimated, and the cultivation method used. The reverse difference, that was seen when comparing yield per m^2 vs. yield per ha, must relate to whether space used for handling is included or not. Yet, space-related issues are less important when using an offshore cultivation sites, as these sites are often less utilized.

The productivity potential of the system used in the Faroe Islands will properly improve over time and does not yet reflect the large scale commercial potential. However, these data are one examples of real world outputs from offshore cultivation and provide a reality check as to what can be achieved in high latitude areas of production.

In theory, the multiple partial harvesting method should reduce the growth rate, because Laminarian species grow the new blade from the meristem between the stipe and the blade using photosynthetic translocation from the old part of the blade to the meristematic sink [45]. Unfortunately, no lines were left as control lines (not harvested) and the effect on growth and yield of multiple partial harvesting still need more investigation.

Multiple partial harvesting was shown suitable in the Faroe Islands, and this can be explained by lower seawater temperatures, and accordingly lower fouling rates. Lower fouling rates, enabled the algae to grow throughout the summer and for several years. Another explanation could be that nitrate was available for macroalgal growth throughout the year in the Faroe Islands [46], and thus the algae could regrow even without the older blade part. Nitrate levels have been measured by the Marine Research Institute on the Faroese continental shelf since 1995 [47], and only three times was the nitrate concentration observed to drop below 3 µM, which is the limiting level for *S. latissima*. The geographical location of the cultivation site at the mouth of a fjord makes the site more comparable to continental shelf conditions than to fjord conditions.

In addition, to favourable nitrate conditions for macroalgal growth, temperature and salinity were extremely stable throughout a year, and

based on Gaard et al. [47] the light-compensation depth measured in a Faroese fjord was below 10 m depth throughout the year except in December and January, where it could be as shallow as 5 m depth. The 10 m long growth lines used in this work are thus above the compensation depth nearly throughout the entire year.

Zhang et al. [27] reported that approximately 90% of the annual blade production was lost due to erosion at the blade tip. A continual multiple partial harvesting could possibly decrease the loss of biomass because the blades were cut when they were still relatively young and with fresh cell tissue. This might also decrease the level of biofouling on the blades because the fouled blade portions infested in spring are removed in July, and therefore not spread to blade tissue newly grown in the interval between July and October.

In addition, the conditions of the offshore cultivation site with high current, large water column, and longer distance to naturally established populations could be the explanation of a very low biofouling density covering the macroalgae.

In comparison with cultivation trials in the North Sea [2,5], we found it possible to cultivate *S. latissima* also through summer and that we could keep the same seeding material over more years. Our findings support the observations of Wegeberg et el. [28] who emphasized the high potential for macroalgal cultivation in the Faroe Islands due to a relatively large ocean area and stable nitrate and temperature levels throughout the year.

Mols-Mortensen et al. [29] found that the quality of cultivated *S. latissima* measured by protein concentration and essential amino acid score was significantly higher in May and June compared to July and August in the Faroe Islands. Other studies have equally shown seasonal variation in the biochemical composition of macroalgae [8,48,49]. Cultivated *S. latissima* biomass that is harvested at different times of the year will thus be expected to show seasonal variation in between batches. This is important to be aware of when harvesting several times during a year.

4.3. Optimised yield

Although the MACR has provided promising results, there is still a need to improve the rig, especially to reduce the handling in relation to harvesting and operational cost. To increase the total production per area sea surface, future constructions could be improved by lengthening the growth lines e.g. to 20 m. This would decrease the production cost per meter of cultivated macroalgae and increase the yield per m^2 sea surface. In the inner Danish seawaters, *S. latissima* can in theory grow down to 12–14 MBSL during the summer period at sites with low light attenuation [50]. In comparison, our findings suggest a maximal cultivation depth of 18–19 m (Table 3). But, the yield at these lower meters is not yet known, and the optimal cultivation depth (length of growth lines) is currently being tested in situ in the Faroe Islands. The final maximal cultivation depth will depend on yield per lowest meter and total cost per meter growth line. The significant variation in growth during different months and at different depths depends on the amount of light throughout the year and the transmittance of light in the water column. Nutrient level and seawater temperature are not likely to play a major role determining seasonal growth performance as these are predominantly constant year-round and seawater masses are fully mixed at the cultivation site. Two factors complicate the calculation of expected yields at different depths. Firstly, cultivated macroalgae will have a shading effect on the underlaying alga and secondly, growth lines will move with the current. As a result, it is necessary to conduct a tentative cost-benefit analyses of cultivation in deeper depths or establish in situ experiments. The linear trend lines (Table 2) showed very low correlation-coefficients ($R^2 < 0.45$) for all harvests. The variation with depth was better described by a 2-grade polynomic trend line (data not shown), because the lines had the lowest yield near surface and at the lowest depths (8–10 MBSL). The decrease of yield with lower depths was proposed by the attenuation of light through water column [50]. The lower yield in the first meters below sea level, was most likely due to interspecific competition between species, as from *A. esculenta* and *L. digitata* which was often found at this upper part of the lines. These two species had thus a better survival at more wave exposed sites, and with occasional high light intensities.

The results show a large variation in the yield between the different growth lines, which is a challenge for large-scale production. One way of improving the production is to increase yield per meter growth line. Uniform sporophyte density and growth are considered important to obtain a higher yield and more stable supply to the market. A way to optimize yield is to understand the seasonal variation in growth and use this knowledge in determining the optimal timing for deployment and harvesting. Selective breeding will also have a major role in optimising yield as seen from Asian macroalgal cultivation and in the agriculture sector. This is therefore an important topic in future research.

Also, the yield and quality are very much dependent on low levels of fouling, and grazing animals. This study demonstrated a very clean biomass of primary food quality, although there was more fouling during the late summer harvest than spring harvest. A mean reduction in expected biomass for third harvest of *A. esculenta* could be due to grazing animals. Understanding the behavior of the grazing animals living in offshore artificial macroalgal fields is therefore of paramount importance. Nevertheless, the offshore cultivation site is considered to contribute towards the low amount of fouling. Our findings support the theory of Arrontes [51], Mols-Mortensen et al. [29], and Bruhn et al. [5] who recommend avoiding sheltered cultivation areas and instead benefit from more open sea areas with stronger currents, lower nutrient levels, and lower and more stable temperatures.

Finally, the business case will be improved by utilizing 100% of the harvested biomass. In such case discarded macroalgae will be used in a zero-waste production and sold as fertilizer, feed and/or be used in the extraction of high value products e.g. fucoidan.

4.4. Cost of offshore cultivation

The cost calculation was based on empirical data generated through the harvest periods 2015 and 2016. From the cost calculations, it was clear that the variable yield (dw per meter of growth line per harvest) had a major influence on the overall economic performance expressed in cost per kg macroalgae. However, the most important finding was the impact of the multiannual harvest on the economic performance, with a 75% reduction of cost per kg cultivated *S. latissima* in the maximum cultivation scenario with six harvests per growth line deployed, compared to one harvest in the base scenario. This means that the capital expenditures (CAPEX) per kg was reduced by factor 2–6 depending on the number of harvests made from the same growth lines without reseeding. This was a result of the very suitable physical conditions in the Faroe Islands combined with offshore cultivation to reduce fouling. In addition, in a large-scale deployment scenario the CAPEX will be reduced as a function of economy of scale related to equipment production and installation.

There was also room for improvements in the operational cost per year (OPEX). The need for innovation in relation to the operations was evident as it remains equal for the various scenarios. The main OPEX cost reduction driver will be increased mechanisation of seeding and harvesting processes. Furthermore, increased know-how through years of operation will reduce expenditures related to inspections of cultivation lines. In addition, an overall improvement of harvested yield per meter or area, e.g. because of a selective breeding program, will reduce the cost per kg of the harvested biomass.

In addition, continuous innovation and climbing learning curves are expected to improve operational efficiency. The improved harvest system using a frame on board to hold the lines was first step to optimize harvesting, however a bigger step will be to develop mechanical harvest equipment and thereby reduce the labour required for harvesting from e.g. three persons to one or two persons. It is important to

bear in mind that the revenue will also be influenced by quality of the macroalgae, both fouling and biochemical composition, which varies with season.

It is expected that the cost will be reduced by economy of scale in large-scale cultivation scenario both in terms of cost of MACR's and in terms of quantity tonnes biomass processed, but the scaling factor will need further investigation. Finally, reducing operating costs by economy of scale in large-scale cultivation scenario, the multiple partial harvesting method, and increasing yield per meter is vital for the commercial viability of offshore macroalgal cultivation.

5. Conclusion

The Macroalgae Cultivation Rig was proven successful for the conditions found at the cultivation location in Funningsfjørður. Next step will be to test the system in another location with similar conditions. The harvest method multiple partial harvesting was proven suitable for *Saccharina latissima* and *Alaria esculenta*, and the method increases the yield per meter growth line in oceans with stable seawater temperature and high nutrient level as in the Faroe Islands. However, to improve the economic feasibility of macroalgal cultivation in the Western world, further efforts are needed both in relation to increasing the yield and lowering operational costs. Future studies should focus on increasing the yield measured as the biomass produced per meter of seeded line. Optimising seeding methods, selective breeding, and longer growth lines (expanding cultivation depth) can all contribute to increased yields, and more research is needed on how to optimize the production in relation to those parameters. The cultivation of macroalgae also requires innovation in relation to lowering the operational costs. In that respect, an important area of focus should be the development of mechanised harvesting methods.

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PAPER II

Offshore seaweed cultivation in the past, present and in future.

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Offshore seaweed cultivation in the past, present and in future

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Running Title: Offshore seaweed cultivation

Abstract

The utilisation of seaweeds is an economically important industry, which is annually worth US$ 6 billion. In the future, the use of seaweeds is expected to increase further in volume and value. Today, seaweeds are mainly produced in Asia using labour-intensive cultivation methods at nearshore, sheltered sites. The need for further increasing seaweed production has raced a challenge for space, and accessible areas are thus more offshore.

To solve these challenges, many offshore cultivation trials have been made in the last fifty years; unfortunately, the developed cultivation structures were not technical and economical viable in open-ocean sites. A seaweed cultivation company in the Faroe Islands has tested a structural design that has many of the solutions to close notable gaps for a future offshore seaweed industry.

This paper describes the challenges of offshore seaweed cultivation, provides an overview of the work that has been made and still is ongoing today. The paper concludes that specific initiatives have promising perspectives for offshore seaweed cultivation and may allow considerable mass production in the near future.

Keywords

Open-ocean, kelp, design, macroalgae, scale.

Abbreviations

CAPEX, capital expenditures; MACR, MacroAlgal Cultivation Rig; OPEX, operational expenditures; ROI, The Return of Investment; TLP, Triple Leg Platform; 2D, two-dimensional; 3D, three-dimensional.

Introduction

Seaweed (macroalgal) cultivation is today a globally economically important industry (Sulaiman Olanrerwaju et al. 2013, Olanrewaju and Magee 2014). While utilization of wild harvested seaweed dates back to the fourth century, novel cultivation methods were first developed in Asia fifty years ago. These cultivation methods allowed a fast development of this aquaculture industry, and today the cultivation of seaweeds counts for 97 % of total global seaweed production (Zemke-White and Ohno 1999, McHugh 2003, Roesijadi et al. 2008, Ferdouse et al. 2017).

In the past ten years, global seaweed production has doubled from 14.7 tonnes wet weight in 2005 to 30.4 tonnes in 2015 (Ferdouse et al. 2017). China is the dominating seaweed producer followed by Indonesia, the Philippines, and the Republic of Korea (Ferdouse et al. 2017).

The future seaweed production has been foreseen to double within ten years (Cottier-Cook et al. 2016, Buschmann et al. 2017, Ferdouse et al. 2017), and this increased demand is driven by a global need for sustainable biomass. Already established markets are for example the use of seaweed as food and in food products, as feed ingredients, and in nutraceuticals. At the same time, new markets are developing, for example, the use of seaweed to produce bioplastics (Roesijadi et al. 2008, Bixler and Porse 2011, Holdt and Kraan 2011, Langlois et al. 2012, Buschmann et al. 2017, Ferdouse et al. 2017).

The fact that seaweed production undergoes global expansion raises new challenges for the industry (Cottier-Cook et al. 2016). In Asia, the challenges are related to accessible space areas nearshore and environmental problems in relation to intensive monoculture cultivation, for example, shading of natural seaweed beds or marine

ecosystem disturbance. In the rest of the world, other challenges are principal for entering the seaweed cultivation industry: 1) coastal areas are highly used for other purposes, 2) space-demanding marine industries are not well recognised (Buck et al. 2004), and 3) the current methods used in Asia are labour intensive and the high salaries in Western countries prevent the industry from being competitive to market price. To avoid these conflicts, the industry aims to move the production into open-ocean environments, and to mechanise and innovate the current methods (Lovatelli et al. 2013, Sulaiman Olanrerwaju et al. 2013).

Offshore seaweed cultivation is expected to have several benefits: 1) no shading of natural seaweed beds, 2) higher growth rate due to increased diffusion rate because of a higher water flow, 3) higher growth rate because of greater light penetration due to higher transmittance, 4) less fouling and epiphytic algal growth, and 5) less grassing.

Many offshore cultivation trials have been made in the last fifty years; unfortunately, most of the developed cultivation structures were either not technically viable or too expensive to be economically feasible. Accordingly, there is a need for developing offshore cultivation structures that are survivable, sustainable and profitable, for ensuring a scalable seaweed industry both in Asia and the rest of the world.

While seaweed cultivation technologies in nearshore, sheltered areas have been developed dramatically in Asia, technologies to cultivate away from shore at large-scale have mainly been investigated in America and Europe. Still, there are challenges to be solved. Until recently, no technical and economical viable cultivation structures for open-ocean areas existed (Roesijadi et al. 2008, Troell et al. 2009, Olanrewaju and Magee 2014, Buck and Langan 2017), but a seaweed cultivation company in the Faroe

Islands has tested a structural design that has many of the solutions to the notable gaps for a future offshore seaweed industry.

This paper describes the challenges of offshore seaweed cultivation and searches for solutions to these challenges from the work that has been made and the work that is still relevant today. To do so, some relevant offshore cultivation structures are described in relation to technical viability, cost of installation and operation, yield performance, and scalability for a perspective on the possibilities for the future.

To be able to compare structural designs and former work, this paper also included a definition of offshore cultivation, a definition of scale, and how to identify Aquaculture Output.

Definition of offshore seaweed cultivation

The term offshore cultivation is understood differently among nations and stakeholders. The term was first used for the production and transmission of electricity, oil, gas and other resources. Hereafter, the term was adopted and widely used in the aquaculture industry but rarely defined in a consistent way (Troell et al. 2009, Lovatelli et al. 2013).

As we already know, the traditional seaweed cultivation in Asia takes place in nearshore and sheltered areas, and before defining offshore we need a definition of nearshore areas. In the review by Roesijadi et al. 2008, nearshore cultivation is defined as being the areas having a sufficiently shallow depth to enable seaweeds to attach and grow or which provide a sheltered environment for aquaculture operations (Roesijadi et al. 2008).

Seaweed cultivation farther off the shore than traditional nearshore areas, can thus be termed off the shore. However, these more offshore sites require some

agreement on the definition (Troell et al. 2009, Sulaiman Olanrerwaju et al. 2013, Froehlich et al. 2017).

In the USA, offshore aquaculture refers to marine farming systems placed more than three nautical miles (NM) from shore, which is the limit for state waters, and within the 200-NM Exclusive Economic Zone (EEZ). This is a clear definition but will not include the sites between traditional nearshore sites and offshore sites according to the USA definition.

Another definition is provided by Sulaiman Olanrerwaju et al. 2013. They work with open-ocean seaweed farming in Malaysia. They define offshore sites to be sites that are exposed to wave heights of 5-10 m, and having a current speed of 100-150 cm/s. Also, the Marine Management Organisation (MMO) in the UK define offshore aquaculture, though using the seawater depth, and they state that every site having a water depth of 50-metres or more is offshore. These defined conditions can nevertheless be present at nearshore exposed sites as well. We therefore need a definition for exposed sites in near distance to shore with offshore-like conditions.

In this work, we use three site categories: The nearshore sheltered, the nearshore exposed and offshore (Table 1). A proposal for the establishment of a definition of these terms is described here using five site attributes: Natural seaweed populations, water depth, maximal wave exposure, and distance to shore.

The offshore site category includes sites where no natural seaweed populations can grow (Roesijadi et al. 2008), where the water depth is 50 meters or greater (Roesijadi et al. 2008, The Marine Management Organisation 2013), where the maximal wave exposure is three meters significant wave height or more (Hagen Rødde et al. 2004), and where the distance to shore is 3NM or more.

The exposed-nearshore site category includes sites with the same conditions as for the offshore sites, but with a distance to shore that is less than 3NM and a location depth of 25 metres or more.

The sheltered-nearshore site category includes sites where there are potentially natural seaweed populations, where the water depths less than 25 metres, where the maximal wave exposure is less than three meters significant wave height, and where the distance to shore is less than 3NM.

The scale of offshore seaweed cultivation and suitable space

Scalability of cultivation structures is crucial for the seaweed industry to grow and definitions of scale are widely used in this industry but rarely defined in a consistent way. A definition of scale is therefore needed.

The operational scale of seaweed cultivation is here based entirely on the annual yield (Table 2) and is divided into four categories with the smallest scale begin an annual yield below 100 tonnes wet weight, and the largest level being an annual yield of more than 100,000 tonnes wet weight.

Aquaculture Output

A standardised method to describe yield is called the Aquaculture Output (AO) and the AO is needed for evaluating yield from a cultivation structure and the related economic feasibility. The AO is widely used in aquaculture but rarely defined in a consistent way for seaweed farming. However, the most used AO for seaweed farming is yield/area/year (tonnes seaweed wet weight/hectare/year; e.g. Fei 2004, Lehahn et al. 2016).

Besides the yield/area/time, the AO can be stated as the yield/metre of seeded rope (kg seaweed ww m^{-1} rope), but this can be of little use when spatial occupation has

importance. When space is included in the AO, the AO can be stated as yield/metre of seeded rope/area (kg seaweed ww m^{-1} rope ha^{-1}).

One could also state AO as operational efficiency/yield/year, including capital expenditures (CAPEX) per year and operational expenditures (OPEX), thus leaving out spatial occupation (cost tonnes $seaweed^{-1}$ yr^{-1}). However, the cost related to spatial occupation will be included in the total cost formulation of CAPEX (e.g. lease of cultivation area) and OPEX (e.g. sailing distance in relation to harvest). The CAPEX has to be expressed as cost per year, calculated from the expected lifetime of the different cost elements of a cultivation rig (cost/lifetime). Furthermore, the cost expression has to be in dry weight biomass, as seaweed species carry different moisture levels. A comparison of AO between different cultivation systems can there be expressed as AO = CAPEXyear/tonnes dw + OPEX/tonnes dw. In addition, the annual tonnes ww/ha will indicate the need for spatial occupational.

Offshore seaweed cultivation trials

To make nearshore-exposed and offshore seaweed cultivation viable, many structural designs were tested at sea during the past fifty years: Ring structures, long-line systems, metal constructions, H-frames, grid systems, and many more. Unfortunately, only a few of these are operative today.

Offshore cultivation structures have been reviewed previously, e.g. by Roesijadi et al. 2008 and Buck and Langan 2017. This paper will not repeat their work but compare structures and include new solutions.

The Marine Biomass Program, USA

The first interest in large-scale cultivation of seaweeds was seen in the USA during the energy crisis of 1970s. Their aim was to find alternatives to petroleum. First research trial with cultivation was carried out with small funding from the U.S. Navy. The research included studies of optimal seeding density, offshore farms structures, and fertilization experiments for *M. pyrifera* (Neushul 1987). A Dynatech report concluded that even with very favourable assumptions the economics were above any practical costs to be considered for energy.

Despite this evaluation, the U.S. Department of Energy funded a new program named the Marine Biomass Program. The aim was to mass cultivate *M. pyrifera* in offshore farms and produce biogas as a replacement for natural gas.

The first farm unit was installed offshore in September 1978. During December the protective curtain came loose, the plants were lost, and the farm was destroyed. A second system was deployed, but the results were again disappointing with little demonstrable success in growing the seaweed for any length of time (Figure 1). By 1981, it became clear that the offshore environment was too harsh. The plan was to

move the operation closer to shore, when the program was terminated in 1983. (Harger and Neushul 1983, Neushul 1987).

While the Marine Biomass Program added considerably to our knowledge base on *M. pyrifera* cultivation, the program was not technically successful (Neushul 1987). Growth data were also collected, but the difficulties of working in the open-ocean environment, of managing seaweed, and extrapolating laboratory data to the field, limited the ability of accurately describe the AO that could have been achieved from a successful operation (Neushul 1987, North 1987).

Survivable BAL's cultivation grid and high average yield, Chile

Chile has a long tradition of harvesting wild seaweed. Today, the three 'wild' *Lessonia* species and *M. pyrifera* are under a strong and increasing pressure of exploitation, mainly for alginate production and as a source of feed for abalone (Buschmann et al. 2014). Recent restriction for exploitation of naturally kelp beds, and the increased demand for biomass, has provided a positive environment for developing a kelp farming industry.

A 3D long-line grid structure was therefore designed (Figure 2), by the BioArchitecture Lab (BAL) and was deployed in three study areas in Chile to test different environmental conditions: Caldera, Ancud and Quenac. During a three-year evaluation period, the best production cycle reached 124 tonnes wet weight/hectare/year (average) in Quenac. The nearshore-exposed site Quenac had the highest productivity also the lowest grassing and fouling pressure. Quenac, had a location depth of 60-metres with a fully mixed water body and lowest sea water temperatures. Caldera, northern Chile, and Ancud, southern Chile, were placed in sheltered and shallow area and experienced high fouling rates and high grassing pressure from sea urchins and

herbivorous crabs. No matter the good results obtained in terms of yield, the system is nowadays not in use mainly because it still cannot compete economically with traditional seaweed gathering from natural beds.

The ring structure installed along with wind mill parks, the North Sea

A series of offshore seaweed cultivation structures were tested in the North Sea in the early 2000'ies seeded with sugar kelp *Saccharina latissima* (e.g. Buck and Buchholz 2004, 2005). The concept of co-use of wind installations and aquaculture was the main driver for the work developed with these offshore structures (e.g. Buck and Buchholz 2004, 2005, Buck and Langan 2017), and one of the structures was a ring that could be integrated with wind mill parks (Buck and Buchholz 2004). The ring construction was modified several times until it reached its final design and resisted the sea condition in the North Sea. The offshore ring device was one of the first structure that survived in an offshore site (Buck and Buchholz 2004). Still, the ring is not used commercially today, properly due to challenges regarding the economically viability of the operation.

The late transplanting strategy to cope with offshore conditions, North Spain

Open-water cultivation in Mataleña, North Spain, carried out in the 2000'ies (Figure 3; Peteiro et al. 2016). Peteiro et al. 2014 had a new approach on offshore farming to try to avoid the harsh winter storms. They tested a transplanting method for *Saccharina latissima* similar to the method used for *Saccharina japonica* in China, where young fronds are deployed during spring instead late autumn; thus, avoiding major storms and torrential rains during the winter. The offshore site was chosen after having serious problems with fish grazing on early young sporophytes between autumn-winter and damage of cultivation structures during winter storms. The 2D cultivation

structure was a square of 50-metre-long horizontal longlines spaced fromeach other with 50 cm, and hold in position at 2-metre below sea surface (Figure 3).

The transplanting method has the advantages of avoiding winter storms, though it creates another problem, namely the continuously annual deployment and picking up of the cultivation structure.

SPAR buoys and H-frame tested offshore, the Netherlands

In the Netherlands, a H-frame structure was designed and tested for offshore cultivation of *S. latissima* by Hortimare in 2012. The concept had SPAR buoys that filled with water when waves became higher, and emptied, when waves calmed down (Figure 4). Several H-frames build from SPAR buoys would hold horizontal lines in a 2D structure. The concept worked in wave basin tests and was thereafter deployed at sea in the offshore site in October 2012. Due to a part of the structure that came loose during deployment, the pipe of the H-frame started to fill up with water and the whole system sunk in January 2013 (Figure 4; pers. comm. Job Shipper, Hortimare; The North Sea Farm Foundation 2018).

Tension-Leg Platform (TLP), Korea

In 2016, the Korea Electric Power Cooperation—Research Institute and Korean Institute of Ocean Science and Technology developed the Tension-Leg Platform (TLP; Figure 5). The TLP system was installed near Cheongsan Island, between Wando and Jeju Island, and several species were grown including *Saccharina japonica*. The TLP utilize the sea in 3D though with a very dense construction that possibly are hard to harvest effectively. The cost for a TLP system was estimated to US$ 500,000 for one-hectare seaweed farm. Although the TLP had potential to grow seaweeds in the offshore environment, the high cost of installation remains an obstacle (Chung et al. 2015).

The Seaweed Carrier and commercial operation, Norway

The company Seaweed Energy Solutions AS (SES) was involved in concepts to upscale seaweed cultivation offshore in Norway since 2010, and SES patented a structure, named the Seaweed Carrier, to enable mass seaweed cultivation in 3D when placed next to each other. The structure was designed as a sheet-like structure with free moving cables with a single mooring on the seabed. The Seaweed Carrier can withstand rough water, has few moving parts, and allows for harvesting (Figure 6; Buck and Langan 2017), but is not currently used commercially.

The MacroAlgal Cultivation Rig, the Faroe Islands

The company Ocean Rainforest Sp/F has since 2010 demonstrated a new cultivation structure that is viable under offshore conditions. The test site can be categorised as a nearshore exposed site with a water depth of 70 meters (Table 3; more information in Bak et al. 2018). The structure is called a Macroalgal Cultivation Rig (MACR; Figure 7) and has proved to withstand the conditions of the North Atlantic Ocean since 2010. Since first deployed, it has been replicated ten times.

The MACR use an approach of vertical growth lines of 10-metres-length and in this way, when structures are placed next to each other, utilize a 3D marine environment opposite of many horizontal long line structures.

One MACR can hold five kilometre of seeded growth line, and occupies a sea surface area of 1 ha, because the fix line is 500-meter-long and has a nominal width of 10 metre on each side. The structure uses no specially designed parts (pers. comm. Bak & Gregersen; Bak et al. 2018).

To make the production profitable, non-destructive multiple harvests is applied, where part of the seaweed blade is left to regrow. This approach allows four-six

harvests over a two-to-three-year period. The multiple partial harvest had a strong impact on the cost (CAPEX) as it reduces the capital cost by 75% (Bak et al. 2018).

Over time, the monoculture of *S. latissima* is taken over by species like *Alaria esculenta* and *Laminaria digitata* take over the line. These species can also be harvested and sold (pers. comm. Bak & Gregersen; Bak et al. 2018)

Discussion

The technical viability (Survivability)

In general, the practice of open-ocean cultivation has never been wide-spread, because of the difficulties of operating under offshore conditions (Roesijadi et al. 2008). In order to successfully grow seaweed offshore or nearshore under "offshore conditions", structures must be sufficiently robust and flexible to withstand severe oceanic conditions. The survivability of a structure is therefore balanced between cost of the structure and its capacity to cope with extreme events, namely its breaking strength. The risk of damaged structures during storms are not only a risk of losing money spent, but it may also cause damage to shipping, shore-side facilities, shoreline development, as well as native habitats (Roesijadi et al. 2008).

Until recently, offshore cultivation has seemed very expensive and technically demanding, but the Faroese developed MacroAlgal Cultivation Rig and the Chilean BAL's cultivation grid have shown promising results in terms of survivability, environmental sustainability, and economy.

The review by Roesijadi et al. 2008 conclude that the failures of early offshore seaweeds farms, were due to equipment failures that were not sufficiently robust to withstand the rigorous conditions. However, there has in our opinion been a trend to over engineer and overthink structures to ensure the survivability, which has resulted in an extremely high cost of installation. For example, the cost of the structures developed in the Marine Biomass Program, the H-frame, and the Triple-Leg Platform. Consequently, the failure of these offshore structures is more likely because of too robust and complex structural designs.

The MACR and the Chilean long-line grid did only use ropes, buoys and anchors in their design. These structures are thus flexible and can move with the oceanic movements - instead of withstanding it. The H-frame and the structures used in the Marine Biomass Program had more vital parts that potentially could break, resulting in a damage of the whole structure, and the special designed parts does also add extra cost to the capital expenditure.

The Spanish method used by Peteiro et al. 2014 avoided cultivation damage by changing the cultivation time-period at sea; though, this has other implications increasing the cost of the operation, which are: once a year deploying and removing the structures, longer hatchery period, and disabling the advantages of multiple partial harvests.

Economic assessments (profitability)

Due to the immature nature of commercial seaweed cultivation there is very little data on economic performance of cultivation systems. Most information and forecasts are based on “excel sheet” calculations rather than actual operational experience with CAPEX and OPEX over a substantial timeseries, e.g. 5-10 years of consecutive operation and the OA during this time. The Return of Investment (ROI) has to be measured as the net value of annual AO compared to the annual cost of invested CAPEX. Such an ROI indicator would provide a useful comparison between different cultivation systems. However, at present stage data is not available for such a comparison.

For comparison we can instead use the average AO of all species cultivated in China which was 97.4 tonnes wet weight/hectare/year calculated by Roesijadi et al. 2008. This can be seen as the “state-of-the-art AO” for seaweed cultivation in nearshore

sites. Future offshore farms should therefore meet this productivity or even surpass it to be competitive.

The following paragraphs give an indication of yields that can potentially be achievable in future large-scale farming (see also Table 3).

An AO of 300 tonnes *M. pyrifera* wet weight/hectare/year were projected by the Marine Biomass Program in USA, though this was never achieved or proved in an upscaled situation. The high extrapolation is possible for *M. pyrifera* because it can be 'coppiced' or partly harvested, which means several sequential harvests and allowing regeneration of plants (Roesijadi et al. 2008, FAO 2016).

In Chile, pilot-production studies with *M. pyrifera* demonstrated an AO of 200 tonnes wet weight/hectare/year could be achieved, though 124 tonnes wet weight/hectare/year was the average yield demonstrated in Quenac (Buschmann et al. 2014, Camus et al. 2016). According to Camus 2018, genetic diversity and breeding studies would increase the yield.

The yield achieved by Peteiro et al. 2014 was 14-16 kg/metre and that gave them an AO of 45.6 tonnes wet weight/hectare/year; approximately, half of what is reached in nearshore commercial farms in Asia. Yield values could be improved by increasing the density of transplanted fronds on the culture ropes.

A future offshore cultivation scenario was made by Fei 2004. They estimated the accessible AO for *Laminaria* species to be 80–120 tonnes wet weight/hectare/year. The extrapolation used data from the well-established nearshore farming and correlate to the "state-of-the-art AO" for seaweed cultivation in nearshore sites in China.

In 2016, the AO of the MACR in the Faroe Islands was 17.6 tonnes wet weight/hectare/year (Bak et al. 2018). Today, the growth lines are placed closer to each

other with one meter in-between. This doubling of meter lines per MACR provide a realistic calculation of the AO, which is 35.2 tonnes wet weight/hectare/year. This is low compared to the other AO extrapolations presented in Table 3, but represent commercial data including harvesting of five kilometres of growth lines. An important fact is that the AO presented here included space for the vessel to operate, and the fact that the seaweed was easily harvested.

The economic assessments of the Marine Biomass Program included comprehensive analyses for offshore seaweed cultivation (Roesijadi et al. 2008), and the Dynatech report concluded that even with very favourable assumptions the economics were above any practical costs to be considered for energy alone.

As well, a feasibility study was made by Camus et al. 2019 of commercial scale *M. pyrifera* production. Their calculations showed that a culture farm of 10 hectares was economically viable, based on the prevailing price of US$ 87/tonne wet weight and a yield over 12.4 kg $m^{-1}y^{-1}$of *M. pyrifera.* These results were completed using a 21-hectare cultivation site with measurements over a three-year period, and the result demonstrated that *M. pyrifera* cultivation in nearshore exposed sites could be commercially viable in southern Chile; indeed, if products with added value were to be developed from the species (Camus et al. 2019)

Bak et al. 2018 made also a cost estimation of the operation in the Faroe Islands using the MACR and multiple partial harvesting (Table 3). The annual CAPEX per tonne dry weight cultivated seaweed was reduced up to 75% by using the multiple partial harvest method enabling four harvests without reseeding. These results were found through a full-scale operation of 5 km of growth lines and with measurements over a two-year period. Additional reduction of CAPEX must be expected through

economy of scale when production is increased and through optimisation of system configuration, e.g. the mooring system and other major cost drivers.

Another aspect of AO and ROI is the ability to increase yield through selective breeding. This will have a direct favourable impact on the ROI.

The reduction of OPEX is mainly related to mechanisation and automatizations of monitoring the cultivation on sea, harvesting and landing the biomass.

Selection of species for offshore seaweed cultivation

Important factors to address when choosing a seaweed species is its productivity (growth rate), the market demand, and sale price. The cultivation methods for seaweeds are greatly variating as different species have different physical requirements (Titlyanov and Titlyanova 2010, Hurd et al. 2014). Therefore, seaweed species are chosen according to the physical conditions of the cultivation site, or a site is chosen according to the requirements of the desired seaweed species. In nearshore-exposed and offshore cultivation sites, seaweed species must be robust enough to withstand a high-energy environment (Buck and Buchholz 2005, Buck and Langan 2017).

Laminarian species and in specific *S. latissima*, which is a native European species, was suggested to be the most robust species to use in offshore mariculture in Europe (e.g. Peteiro et al. 2006, Buck and Langan 2017, Kerrison et al. 2018). *S. latissima* has a relatively high growth-rate and seeding techniques for this species is well (Peteiro et al. 2006).

In the Pacific Ocean, *M. pyrifera* was suggested for cultivation being a robust seaweed species with a high growth rate (North 1987). Under optimal growth condition, some *M. pyrifera* can produce almost a kilo of wet weight per day (Harger et al. 1983).

Large-scale scenarios

From large quantities of seaweed, it could be possible to meet some of the major challenges the world is facing, for example food supply and displacing fossil fuels with renewables (Lehahn et al. 2016). But where are the suitable sea areas?

An large-scale scenario was presented by Roesijadi et al. 2008. They stated that it is possible to produce 3.5 billion tonnes dry weight/year more food produced from seaweed, which is more than half of the food produced today from land and sea. The spatial area used for this was 1% of the Earth surface. To make this calculation, several assumptions was made. For example, they used the yield of traditional nearshore seaweed cultivation, because real numbers of offshore production did not exist at that time.

Today, more valid data of yield from offshore farming was made by Bak et al. 2018 and Camus et al. 2018 when cultivation S. latissima and M. pyrifera in nearshore sites exposed to open-ocean conditions. Using these numbers (Table 3), the area needed for the same production would be 13.9 million km^2 when growing *S. latissima* using the MACR and would be 3.9 million km^2 when growing *M. pyrifera* using the BAL's cultivation grid.

Scalability and consequences

The MACR has been replicated 10 times and it is easily scalable, though it is space demanding compared to other structural designs. Therefore, the marine spatial planning become more vital for an increased production scenario.

Potential consequences of offshore cultivation of seaweeds are the interference with marine navigation, including shipping, commercial fishing, and recreational

boating (Roesijadi et al. 2008). Conflicts between aquaculture operations and other coastal uses are common and generally revolve around competing use of space.

At the cultivation site in the Faroe Islands there are many other marine activities in the same fjord: Salmon farming, lobster catching, boat trafficking, tourism, and whale catching. Nevertheless, thus MACR allow smaller vessels to pass the structures without creating damage or tangling of the seaweed lines. This is due to the flexibility of the structural design, where lines can move like a "kelp", as it is only attached to the fix line at 10-meters depth. Lobster catching and fishing can, therefore, occur in the cultivation area without interfering with the seaweed cultivation. The cultivation site could even be a good spot for divers and tourism to explore the biodiversity that surround the cultivation site.

A consequence of large-scale cultivation is that the seaweed stock slows down water movement. This will increase the demand for high water flow for adequate diffusion of gases and dissolved substances to the seaweed. Only in rapidly flowing water, will the productivity of seaweeds be maximized. Under open-ocean conditions this will rarer be the situation as current is normally present. The structural design will need to consider this issue and spacing between units may be needed.

Moreover, intensive seaweed cultivation can encourage disease organisms to flourish. Poor environmental conditions lead to increased disease susceptibility, which make the necessity of selecting the right growing areas more urgent (Roesijadi et al. 2008, Buschmann et al. 2014). Diseases have occurred in the Philippines during 2011-2013 where genetically identical seaweed stocks were infected (Ferdouse et al. 2017). This must be prevented in the best way through clever breeding, fallow, and by leaving

empty spaces between fields of seaweed**.** Titlyanov and Titlyanova 2010 has suggested some guidelines on how to prevent disease.

Sustainability

Seaweed farming is considered as the least environmentally damaging form of aquaculture and is often described with multiple positive effects (Troell et al. 1999, Titlyanov and Titlyanova 2010, Buschmann et al. 2014, Duarte et al. 2017). Nevertheless, traditional seaweed farming has taught us the importance of making a sustainable production to preserve the environment and the resources of the Earth.

Algae perform about half of the global carbon fixation on Earth (Chung et al. 2011, Duarte et al. 2017), and may also account for much of global biological carbon storage, thereby being a natural means for the reduction of greenhouse gas emissions (Roesijadi et al. 2008). By sequestering carbon dioxide and increasing pH in seawater, seaweeds could thus play a significant role in reducing carbon emissions and ocean acidification (Clements and Chopin 2016, Buck and Langan 2017)**.**

Both natural seaweed beds and cultivated seaweeds take up nutrients and provide nutrient cycling (Manninen et al. 2016, Buschmann et al. 2017). When seaweeds are cultivated in eutrophic sea areas, they provide bioremediation.

In Asia, the large-scale seaweed cultivation in shallow waters has been so effective in using the nutrients that fertilization was required to increase productivity. Regrettably, the fertilization has resulted in ecosystem disturbance and lost biodiversity. To avoid this situation, marine spatial planning should be carefully used when large-scale seaweed fields are to be established.

Nutrient levels in the sea and over a year is nevertheless site-specific, and for most offshore or exposed-nearshore sites water masses pass the seaweed constantly and

bring enough nutrients to the crop. However, in some offshore sites, like those investigated in the Marine Biomass Program or tropical regions, there will be times of year where nutrients are limited.

As well as capturing carbon and taken up nutrients, these artificial ocean forests create an additional habitat and provide food for a diverse array of fish and invertebrates of conservation importance (Peteiro et al. 2014, Buschmann et al. 2017). Large-scale seaweed farms will host a diverse group of species and support biodiversity (North 1987, Skjermo et al. 2014, Buschmann et al. 2017).

In the Faroe Islands, growth lines are left in the ocean for 3-4 years and create a diverse ecosystem over time. This community will increase species abundance locally, even though initial intended as a monoculture. Appropriately designed cultivation structures can thus serve as additional communities and provide ecosystem services that needs to be valued appropriately(Chopin 2014, Buck and Langan 2017)

Evaluation of ecosystem goods and services of natural seaweed ecosystems has been summarized in several publications, see Buschmann et al. 2017, but has not been sufficiently evaluated for offshore seaweed cultivation. The reason for this is of course absence of such operations, but also a lack of understanding and appreciation of what these communities can provide. Useful references could, for example, be the Australian kelp forests that cover ~71,000 km^2 of the southern coast and generate an estimated value of US$7.7 billion annually (~US$110,000 km^{-2} yr^{-1}) (Buschmann et al. 2017). Another example, with an even higher value estimated, was made by Costanza et al. 2014. Here the seaweed forests were estimated to provide an ecosystem service of a value of US$2,891,600/$km^2$/year.

The large-scale seaweed cultivation in Asia has been harmful to the local benthic ecosystems, as the intensive mono-cultivation takes place in shallow-water lagoons, small bays, and estuaries (Titlyanov and Titlyanova 2010, Buschmann et al. 2014). The impact of offshore cultivation on the benthic community is still unknown, but preliminary environmental studies at the 21-hectare pilot-farm installed in southern Chile indicated that no benthic modifications were seen during three years of cultivation (Buschmann et al. 2014).

The research team behind the Marine Biomass Program in California stated the need for growing seaweed in oceanic sites, where even the giant kelp could not grow naturally (North 1987). The use of offshore or nearshore-exposed sites, using the definition stated in this paper, avoid out-competing natural seaweed beds, seagrasses, and corals. This is crucial for the industry to grow in a sustainable way (Roesijadi et al. 2008, Titlyanov and Titlyanova 2010). Our recommendation is that all future seaweed farming should use marine sites that fulfil the definition of being nearshore exposed or offshore to avoid interference with natural seaweed beds. Marine spatial planning seems to be the most adequate tool to address this issue (Lester et al. 2018).

Finally, offshore and nearshore-exposed seaweed installations do need a design respecting marine mammals and birds. That implies that fish, mammals, and birds can pass the structures without being tangled (Roesijadi et al. 2008). The MACR is being operated in an area with many seabirds, seals, and pilot whales; nevertheless, no complications have occurred since first structure was deployed (pers. comm. Bak & Gregersen). Accordingly, it is possible to design cultivation structures in respect to the wildlife.

Conclusion

In conclusion, the utilization of the ocean for large-scale seaweed cultivation is today more likely than ever before. Until recently, offshore cultivation has seemed very expensive and technically demanding, but the Faroese developed MacroAlgal Cultivation Rig and the Chilean BAL's cultivation grid have shown promising results in terms of survivability, environmental sustainability, and economy.

A production scenario with the goal to increase the global food production by 50%, producing 3.5 billion tonnes dry weight seaweed per year, would require a sea area of 3.9-13.9 million km^2 (~0.7-2.6 % of Earth surface) depending on the system and species used. This calculation was based on the work made by Roesijadi et al. 2008 but included data from commercial-scale nearshore sites exposed to open-ocean conditions.

The MACR shows the lowest Aquaculture Output, as the structure include space for handling the lines, however this is the only system in operation today deployed under offshore conditions. Other initiatives have also proved their technical viability, but their tests have mostly been restricted to short test-periods and has faced the challenges of handling and harvesting the seaweed.

Upscaling of the MACR and the BAL's cultivation grid still needs to be proven, though multiple partial harvests and structural optimisation has lowered the cost noticeably. The cost of production can be further lowered from mechanization of the operation, through selective breeding, and bio-refinery processes.

Despite the remaining challenges, the ultimate potential in terms of biomass production is so high that continued investigation in the MACR and other structures is warranted.

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Figures legends

Figure 1. The U.S. Navy researched an approach to growing kelp in the open ocean. In the early 1970s the structures where anchored modules like an upside-down umbrella with large buoys at each corner, using cold-water upwelling pipes placed in the middle. Growth lines were held at a depth of 12-15m on a grid system and partly seeded (1 plant per $10m^2$). Plants were planned harvested after 4 years, when sufficiently self-seeding had occurred (North 1987). *width of one column (80 mm)*

Figure 2. BAL's cultivation grid in Chile. Pilot scale cultivation of *Macrocystis pyrifera* in a 21-ha plot. Yield reached and average of 124 Ton ww ha-1 yr-1 during a 3-yr. period. A grid composed of 21- 100 x 100 mt modules, with 99 -100 m. lines each. More than 20 seeded cohorts were followed in time to determine the best production conditions (Camus et al. 2016). *width of one column (80 mm)*

Figure 3. Using the Asian approach in Spain with late transplanting method in an open ocean site. The cultivation structure was a square having horizontal longlines, hold at 2-metre below sea surface (Peteiro and Freire 2009). *width of one column (80 mm)*

Figure 4. The H-frame had a total length 120m and was 10m wide and was designed to sink when harsh weather appeared. The other structure consisted of a set of steel cables, which was submerged two meters below the sea surface and was held by anchors and floating buoys. In between, horizontal nets (10m x 10m), seeded with sporophytes, and suspended between those cables (The North Sea Farm Foundation 2018). *width of one column (80 mm)*

Figure 5. Mass production of seaweed biomass in Korea using the Tension-Leg Platform (TLP). The TLP occupied an area of four hectare having nine main buoys and nine anchors. Between main buoys a grid system of longlines were hold in position by smaller float. From the longlines vertical growth lines were attached (Chung et al. 2015). *width of one column (80 mm)*

Figure 6. A sheet-like structure with free moving cables with a single mooring. The flexible hybrid long-line has carriers of 6.5m length and 5m deep as a 2D flexible units. In this long-line system the single long-line operates as a backbone for 20 of the carriers. Survivable in rough locations and has low cost due to few moving parts. Harvesting and handling is easy. Manually harvested. Patented seaweed carrier system by SES, Patent No. EP 09 836 439.1. (Seaweed Energy Solutions 2018). *width of one column (80 mm)*

Figure 7. Nearshore-exposed cultivation site in Funningsfjordur, the Faroe Islands (top left), the MacroAlgal Cultivation Rigs placed side by side (down left), the operation vessel with crane used to lift seaweed lines (top right), and the structure design of the MacroAlgal Cultivation Rig (down right): The design consisted of a 500-m long fix line (C) suspended horizontally at 10 MBSL. Two main surface floats (D) were connected to the fix line and 40% submerged in a static state. The mooring system consisted of four 120-m anchor lines, which were attached to the fix line and anchored to the seafloor with 1–1.5 t steel anchors (E). The rig had approximately 500 growth lines (B) of 10-metre length attached to the fix line with a float fixed at the opposite end, stretching the lines in a vertical position (Bak et al. 2018). *width of one column (80 mm)*

Tables

Table 1. The three seaweed cultivation categories for site definition and the four site description parameters. *width of one column (80 mm)*

Categories	**Natural seaweed**	**Water depth**	**Max. sign. wave exposure**	**Distance from shore**
Offshore	No	≥ 25m	> 3m sign.	> 3NM
Nearshore Exposed	No	≥ 25m	> 3m sign.	< 3NM
Nearshore Sheltered	Yes	< 25m	< 3m sign.	< 3NM

Table 2. Operational Scale Definition of seaweed cultivation. *width of one column (<80 mm)*

Scale	**Seaweed yields (tonnes ww year^{-1})**
Micro	<100
Small	100-10,000
Medium	10,000-100,000
Large	>100,000

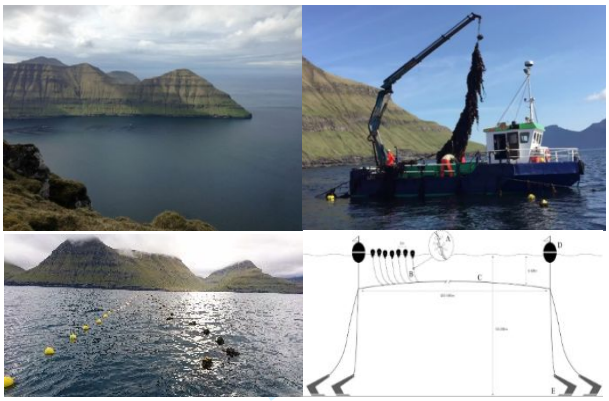
Table 3. An overview of seaweed cultivation structures. *Full page or half page*

Structure name / project name	Origin country, location	Test period	Site Cate-gory	Site description					Unit size tested at sea (ha)	AO (T ww ha^{-1} $year^{-1}$)	Yield (kg ww m^{-1} rope $year^{-1}$)	Species	No. of years tested at sea	In operation today	Cost (US$)	Reference
				Natural seaweeds	Location depth (m)	Max. sign. Wave height	Max. Current	Distance to shore (km)								
The Marine Biomass Program	USA, California	1970-1983	N.E.	No	150	-	-	~1	0.48	55-300#		*MP*	<1	No	CAPEX: 570 million OPEX: 61.4 million yr^{-1}	(Harger et al. 1983, Neushul 1987, Neushul et al. 1992)
BAL's cultivation grid	Chile, Quenac, Caldera & Ancud	2010-2013	N.E.	No	60	3	115	-	21	124*	Mean 12.4*	*MP*	3	No	CAPEX: 6,000 ha^{-1} OPEX: 8,000 ha^{-1}	(Buschmann et al. 2014, Camus et al. 2019)
Offshore Ring System	Germany, North Sea	2004-	O	-	100	-	-			~109 T dw $year^{-1}$		*SL*	-	No		(Buck and Buchholz 2004, 2005, Buck et al. 2004)
A culture raft	Spain, Mataleña	2000-2008	N.S.	-	~20	0.5-3	48-92	~1	0.12	45.6#	Max. 16*	*SL UP*	<1	No		(Peteiro et al. 2014, 2016)
The H-frame and SPAN buoys	The Netherlands, Texel	2012-2016	O	No	100	7-8	120	12	0.04	-		*SL LD*	<1	No		(pers. comm. Hortimare 2018, The North Sea Farm Foundation 2018)
The Tension-Leg Platform (TLP)	Republic of Korea, Jeju Island	2010-2012	O / N.E.	-	-	-	-	-	4	150-300#	Max. 80.6	*SJ*	2	?	0.5 million ha^{-1}	(Chung et al. 2015)
The Seaweed Carrier	Norway, Trondheim	2009-	N.E. / N.S.	-	?	?						*SL*	-	?		(Seaweed Energy Solutions 2018)
Macro Algal Cultivation Rig	The Faroe Islands, Fun-ningsfjørdur	2010-	N.E.	No	70	4	25	0.5	1	35.2*	Mean 5.8*or 58 m^{-2}	*SL AE LD*	8	Yes	CAPEX: 13,364 ha^{-1} OPEX: 10,676 ha^{-1}	(Bak et al. 2018)

Readers instructions: Offshore = O, nearshore-exposed = N.E., and nearshore-sheltered = N.S. Aquaculture Output = AO. All estimated numbers/goals are marked with a hash-tack (#), and all tested numbers are marked with a star (*). *Macrocystis pyrifera* = MP, *Saccharina latissima* = SL, *Saccharina japonica* = SJ, *Laminaria digitata* = LD, and *Alaria esculenta* =AE, *Undaria pinnatifida* = UP. Capital Expenditure = CAPEX, Operational Expenditure = OPEX.

Graphical Abstract

Use figure 7 as the graphical abstract (picture 7a, 7b, 7c and 7d).



Text: The MacroAlgal Cultivation Rig is survivable and scalable offshore conditions in the North Atlantic Ocean.

Author Biographies

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Urd G. Bak is working as industrial researcher at Ocean Rainforest. She has a MSc in Environmental biology and Geography. Currently, Urd is completing her Industrial PhD at the National Food Institute at DTU. The field of her work is an investigation of the nutritional composition of cultivated macroalgae and how to optimize seeding and cultivation methods. Over the past 10 years, she has worked with seaweeds in relation to commercial production from seeding to harvesting and investigated the nutritional composition with focus being on the species *P. palmata*, *A. esculenta* and *S. latissima* cultivated offshore in the Faroe Islands.

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Javier Infante is currently the CEO of Patagonia Seaweeds SpA, a company dedicated to consultancy in seaweed aquaculture. He is an Aquaculture Engieneer, with a MSc. In Bussines Administration. He has more than 10 of hands-on seaweed cultivation experience at the hatchery and ocean level. Recent projects in which he has taken a lead are mostly related to seaweed culture for the biofuels and phycocolloids industries, as

well as social oriented technology transfer projects. His latest work has been advocated to techno economic assesments of seaweed cultivation in Chile and United States.

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Ólavur Gregersen is Co-founder and Managing Director of Ocean Rainforest with the responsibility for the overall project management and business development of Ocean Rainforest. He has more than 20 years of experience as entrepreneur and international consultant as well as non-executive Director in several innovative companies and projects. Ólavur has specialised in business development, project management and socio-economic impact analysis and he has been the coordinator of several European and Nordic research projects like WhiteFishMaLL, MacroValue and MacroBioTech. The project topics cover macroalgal cultivation and processing, marine ecosystem management, information and communication technology and sustainable food and feed production.

LAYOUT INSTRUCTIONS

Figure 1.

A B

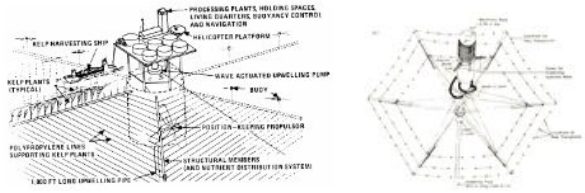
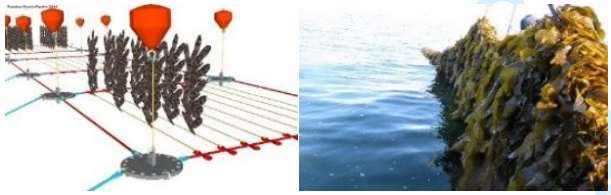


Figure 2.

B A



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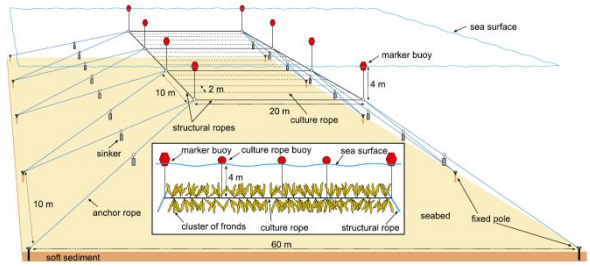

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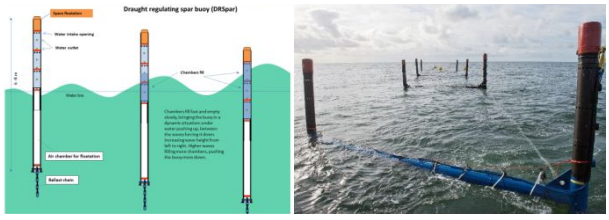


Figure 5.

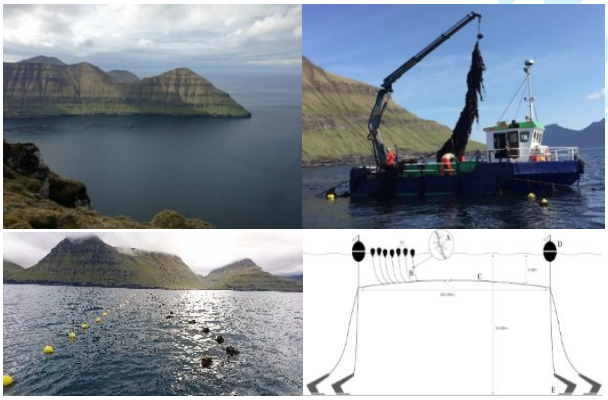
B A

Figure 6.

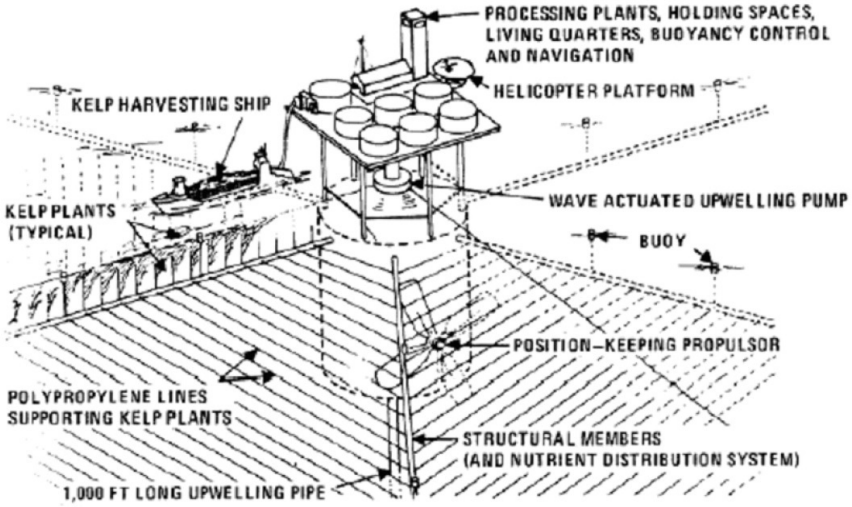
B A

Figure 7

A B



C D



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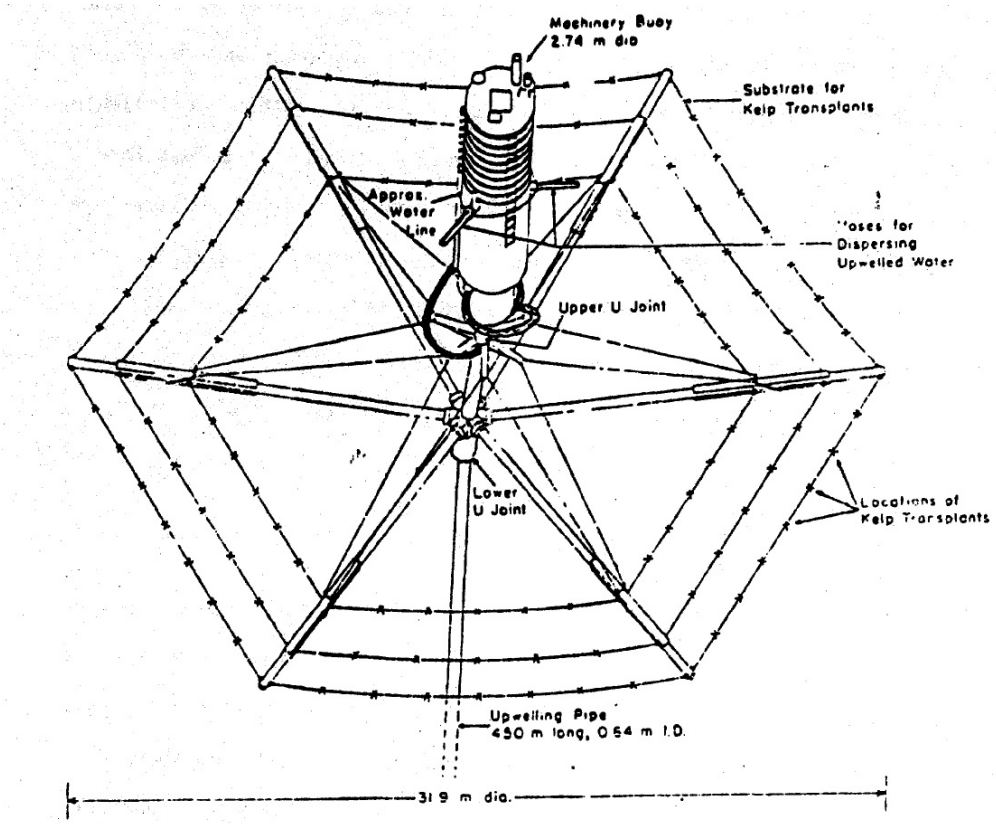

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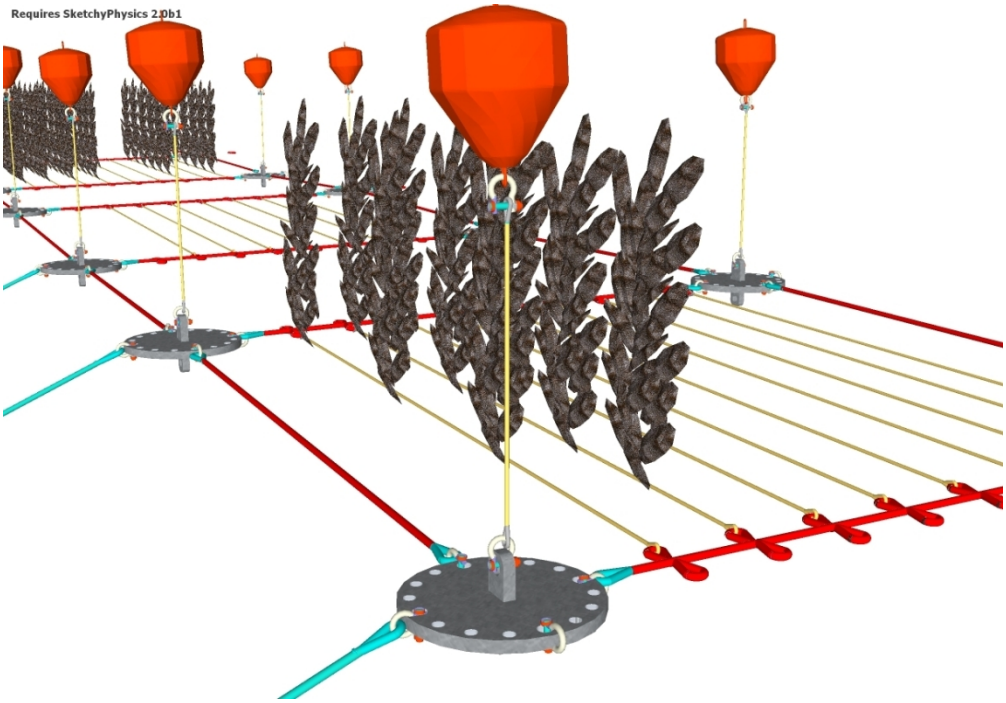

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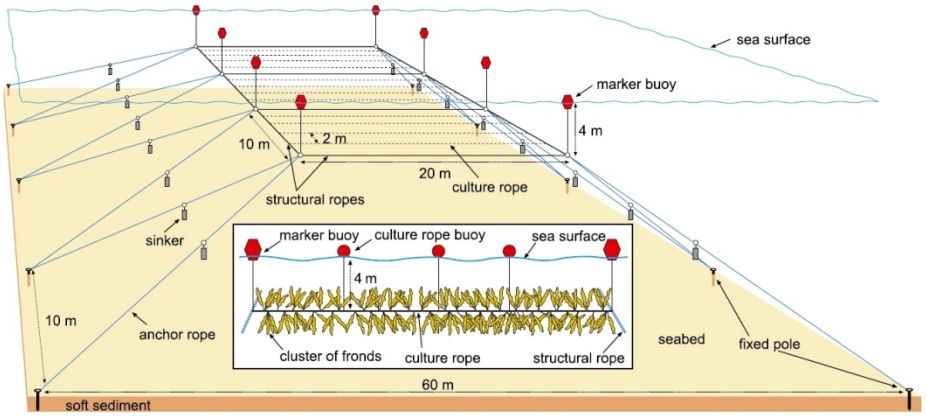

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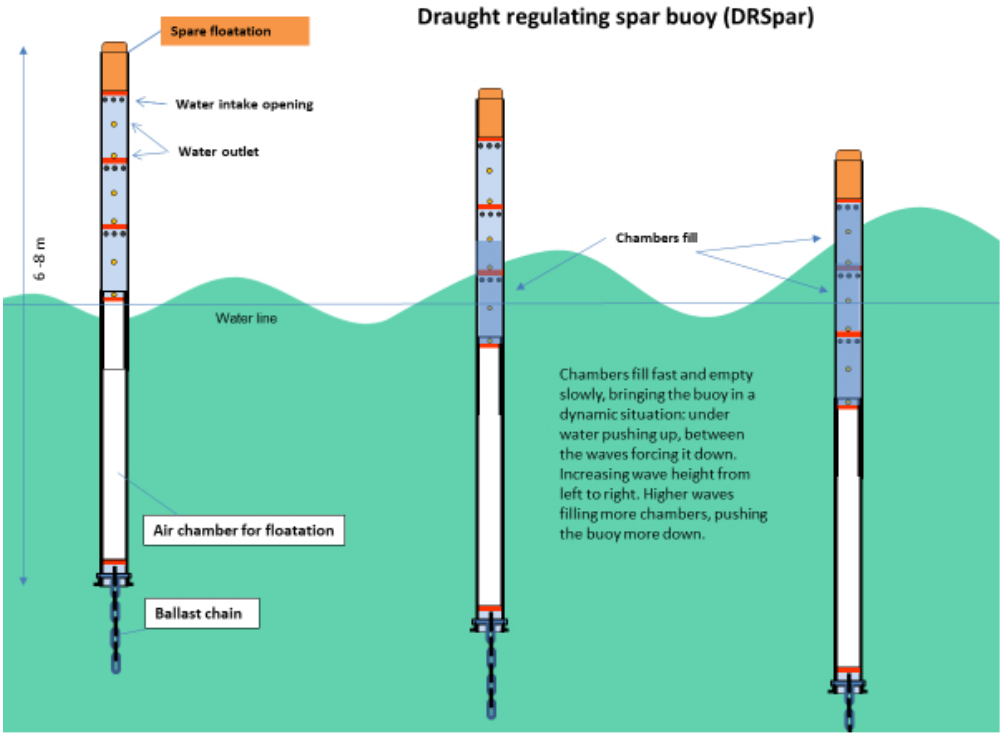
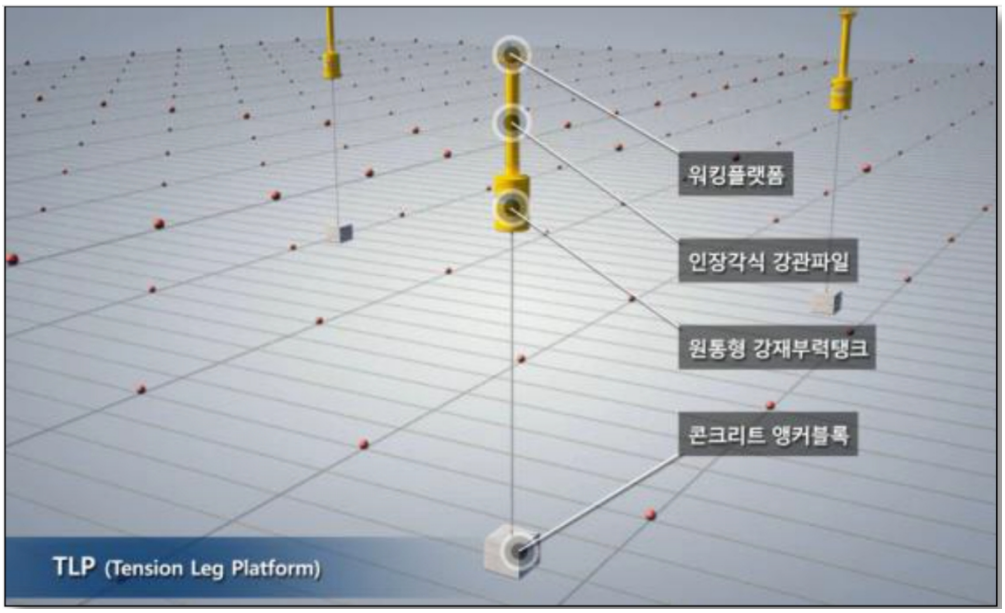

Draught regulating spar buoy (DRSpar)
Spare floatation
Water intake opening
Water outlet
6 -8 m
Water line
Chambers fill
Air chamber for floatation
Ballast chain
Chambers fill fast and empty slowly, bringing the buoy in a dynamic situation: under water pushing up, between the waves forcing it down. Increasing wave height from left to right. Higher waves filling more chambers, pushing the buoy more down.


워킹플랫폼
인장각식 강관파일
원통형 강재부력탱크
콘크리트 앵커블록
TLP (Tension Leg Platform)

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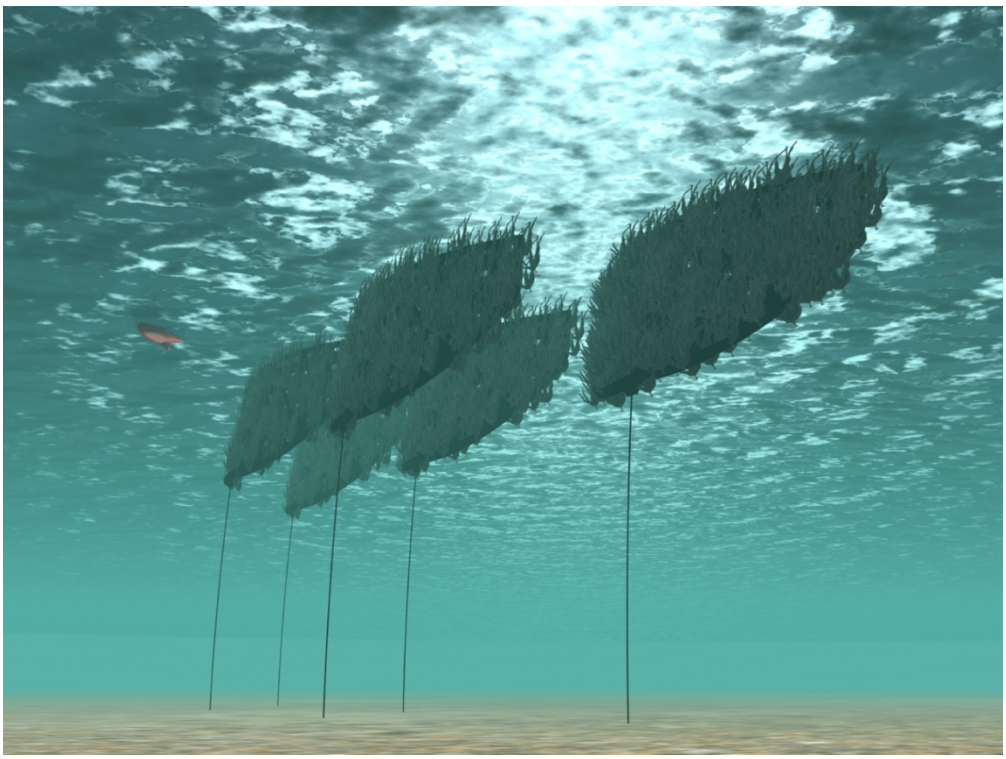

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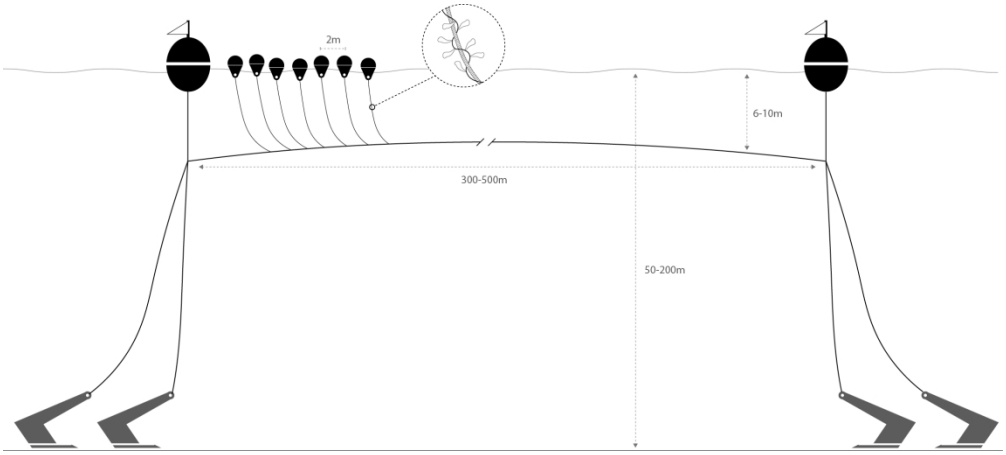

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PAPER III

The seasonal variation in nitrogen, amino acid, protein and N-to-Protein conversion factors of commercially cultivated Faroese *Saccharina latissima* and evaluation of the use for food and feed.

Urd Grandorf Bak, Cecilie Wirenfeldt Nielsen, Gonçalo Silva Marinho, Ólavur Gregersen, Rósa Jónsdóttir & Susan Løvstad Holdt

Algal Research, submitted (Oct 2018), resubmitted (Feb 2019)

Manuscript Details

Manuscript number	ALGAL_2018_814_R1
Title	The seasonal variation in nitrogen, amino acid, protein and nitrogen-to-protein conversion factors of commercially cultivated Faroese Saccharina latissima and evaluation of the use for food and feed
Article type	Full Length Article

Abstract

The demands of new food sources are increasing with the increasing human population on earth. Proteins are a main nutrient for human consumption and in animal feed, which will be in short supply in the near future. Many macroalgal species have shown to possess significant levels and quality of protein, comparable to conventional protein-rich foods. The brown macroalga Saccharina latissima was commercially cultivated in an open ocean area in the Faroe Islands. The effect of depth, cultivation site and seasonal variation in nitrogen, protein concentration, and the amino acid profile were investigated to study the potential of Faroese cultivated S. latissima as a protein source. Moreover, the nitrogen-to-protein conversion factor was calculated. The average nitrogen concentration was 2.1±0.2% of dry weight (dw) with no significant variation between sites, a single month with significant variation between cultivation depths (March 2016), and a significant seasonal variation (among most months). The average protein concentration determined by summing up total amino acids was 4.3±0.9% of dw, and comparable to or slightly lower than other studies. There was no depth, site or seasonal variation in AA-protein concentration for the cultivated S. latissima. The lack of seasonal variation was most likely a consequence of the year-round stable physical conditions in the Faroe Islands, and compared with other studies surprising as most found seasonal variation of AA-protein. The quality of the protein was high (EAA score >100%) in March, although the low total concentration of protein limits the possibilities to use S. latissima solely as a protein source or for protein extraction and other nutrients should be investigated to understand its potential as a food or feed source. This study will recommend estimating total protein concentration by summing up the total amino acids (AA-protein), as the widely used 6.25 factor is highly overestimating the protein concentration.

Keywords	Seaweed; macroalgae; kelp; seasonality; offshore; biochemical composition.
Taxonomy	Applied Sciences, Aquaculture Nutrition, Algal Culture, Food Composition, Nutrient, Proteins in Food
Manuscript category	Biochemical Characterization

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Suggested reviewers	Michele Stanley, Mette Nielsen, 3. Joël Fleurence, Stephen Bleakley Bleakley

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request

Sunday, October 14, 2018

To: Editor of Algal Research

Dear Editor,
Enclosed please find the manuscript, entitled:

"The seasonal variation in nitrogen, amino acid, protein and N-to-Protein conversion factors of commercially cultivated Faroese Saccharina latissima and evaluation of the use for food and feed"

for publication in Algal Research submitted by Urd Grandorf Bak, Cecilie Wirenfeldt Nielsen, Gonçalo S. Marinho, Rosa Jonsdottir, Olavur Gregersen, and Susan Løvstad Holdt. This manuscript is new, is not being considered for publication elsewhere, was not previously submitted to Algal Research and is the original work of the authors. All authors agree that this manuscript should be submitted to Algal Research.

Novelty: This work serves as state-of-the-art investigation of chemical composition of cultivated seaweed, here *Saccharina latissima*, when farmed in the Faroe Islands. For the first time an offshore cultivation site (>50m water depth) was used in comparison with a moderate wave exposed site, and for the first time samples were taken from the same lines (and algae) in a 2-year period, without re-seeding the lines. The protein content was estimated from total amino acids and the amino acid profile was described. Also, the site, depth and seasonal variation in total nitrogen content was analysed, and content was compared to previous studies of *S. latissima* nitrogen content investigated for other cultivation locations in Europe. Also, the widely used convention factor of 6.25 was discussed and found to be overestimating the real protein content of *S. latissima*.

Next page contains the author contribution form.

Yours truly,
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Sunday, October 14, 2018

AUTHOR CONTRIBUTIONS FORM

Manuscript title:
The seasonal variation in nitrogen, amino acid, protein and N-to-Protein conversion factors of commercially cultivated Faroese *Saccharina latissima* and evaluation of the use for food and feed

Journal:
Algal Research

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Susan Løvstad Holdt (SLH)

Conception and design: UGB, SLH and OG

Collection and assembly of data: UGB and OG

Analysis and interpretation of data: UGB, CWN, GSM, RJ, and SLH

Drafting of the article: CWN, UGB, GSM, and SLH

Critical revision of the article for important intellectual content: All authors

Final approval of the article: All authors

Highlights

- Offshore cultivated Faroese sugar kelp had no seasonal variation in protein content
- Faroese sugar kelp harvested in spring had a high essential amino acids score (>100)
- Sugar kelp is a high-quality protein though low quantities source for food and feed
- Conventional conversion factor (6.25) overestimate the protein content in sugar kelp

Title

The seasonal variation in nitrogen, amino acid, protein and nitrogen-to-protein conversion factors of commercially cultivated Faroese *Saccharina latissima* and evaluation of the use for food and feed

Authors

- Urd Grandorf Bak[1,2]
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- Gonçalo Silva Marinho[1]
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Keywords

Seaweed; macroalgae; kelp; seasonality; offshore; biochemical composition.

Abbreviations

MBSL, meters below sea level; dw, dry weight; ww, wet weight; AA, amino acids; TAA, total amino acids; EAA, essential amino acids; AA-protein, protein determined by summing up TAA.

Pages with colours

Fig. 2 and 6 will need to be printed/published in colours.

Abstract

The demands of new food sources are increasing with the increasing human population on earth. Proteins are a main nutrient for human consumption and in animal feed, which will be in short supply in the near future. Many macroalgal species have shown to possess significant levels and quality of protein, comparable to conventional protein-rich foods. The brown macroalga *Saccharina latissima* was commercially cultivated in an open ocean area in the Faroe Islands. The effect of depth, cultivation site and seasonal variation in nitrogen, protein concentration, and the amino acid profile were investigated to study the potential of Faroese cultivated *S. latissima* as a protein source. Moreover, the nitrogen-to-protein conversion factor was calculated.

The average nitrogen concentration was 2.1±0.2% of dry weight (dw) with no significant variation between sites, a single month with significant variation between cultivation depths (March 2016), and a significant seasonal variation (among most months). The average protein concentration determined by summing up total amino acids was 4.3±0.9% of dw, and comparable to or slightly lower than other studies. There was no depth, site or seasonal variation in AA-protein concentration for the cultivated *S. latissima.* The lack of seasonal variation was most likely a consequence of the year-round stable physical conditions in the Faroe Islands, and compared with other studies surprising as most found seasonal variation of AA-protein.

The quality of the protein was high (EAA score >100%) in March, although the low total concentration of protein limits the possibilities to use *S. latissima* solely as a protein source or for protein extraction and other nutrients should be investigated to understand its potential as a food or feed source.

This study will recommend estimating total protein concentration by summing up the total amino acids (AA-protein), as the widely used 6.25 factor is highly overestimating the protein concentration.

1. Introduction

New food sources are essential to investigate since common food and feed source will be in short supply in the future as a result of the growing world population [1]. Proteins are essential building blocks of all living organisms and new protein sources are to be investigated. Many macroalgal species (seaweeds) have shown to possess significant levels of protein quantity and quality. In some cases even higher (up to 47%) than conventional protein-rich foods in dry forms [2–7].

In Asian countries, macroalgae have been a great tradition as a human nutritional source. Macroalgae can be utilized either directly consumed or as a food ingredient, such as thickening agents, or in animal-feed as alternative high-quality proteins [1,3,8–10]. The macroalgal industry is growing and more than 27 million tonnes were harvested worldwide in 2014 with the estimated value of US$ 5.6 billion [5].

The quality of protein for human consumption depends on the profile of the amino acids and the digestibility of the proteins [1,11]. The protein requirements that meet the metabolic needs for human populations have been published by WHO, FAO and UNU [12], and protein sources can be pronounced using an essential amino acids (EAA) score. The protein concentration in macroalgae varies according to species but is also influences by extrinsic factors such as seasonal variation and the changes in physical factors e.g. light and nutrient availability [13].

Protein concentration is generally higher in Rhodophyta (up to 47% of dw) and Chlorophyta (10-25% of dw) than in Phaeophyceae (5-13% of dw) [3,14]. However, the cultivation and availability of the large brown kelp species (in the order Laminariales) are more likely to be cultivated in large quantities in future in Europe. This is due to their known life-cycle which allows sexual reproduction and large-scale seeding. The kelp species have in general a high growth rate and a large yield per meter of cultivation line [5,15], and can most likely be automated easier than the red and green species of macroalgae.

A growing interest is seen in Europe for the kelp species *Saccharina latissima* (Linnaeus) C.E. Lane, C. Mayes, Druehl & G. W. Saunders, commonly known as “sugar kombu” or “sugar kelp”, cultivated in commercial monoculture or in integrated multi-trophic aquaculture [9,16]. *Saccharina latissima* is an

edible species, which is accepted as food (non-novel food) in EU, and thus is suitable for consumption and further commercialisation. The seasonal variation of biochemical compounds, including proteins, has however been addressed as a major challenge for commercialisation and the year-round-supply of same quality biomass. Consequently, a concentrated effort has been addressed in the past years to increase data and knowledge about the possible seasonal variations in the composition of cultivated and wild harvested *S. latissima* for better planning of optimal harvest time, use of the biomass in end-products and in cascading biorefineries [2,4,9,17–21].

Several studies are currently questioning the general approach for the analysis of protein concentration in macroalgae. The traditional nitrogen-to-protein (N-to-protein) conversion factor of 6.25, used to find the crude protein concentration, is based on the assumption that samples contain protein with 16% nitrogen and an irrelevant amount of non-protein nitrogen such as nitrate, nitrite, and ammonia [22]. This assumption is invalid if the material consists of high amounts of other nitrogen sources [23].Therefore, a current trend has been to calculate the specific N-to-protein conversion factor for the specific foodstuff because using conversion factors is a practical method [23–26].

S. latissima is been cultivated on a commercial scale (~10 ha) at an open ocean location in the Faroe Islands. The temperature in the Faroese waters is almost stable (7-11°C) throughout the year [27,28] and nutrients are sufficient for growing *S. latissima* all year round [29,30]. This should make the environmental growth conditions optimal for macroalgal cultivation, and promising growth and yield potential have also been observed for *S. latissima* in the Faroe Islands [15,21,29]. The Faroe Island cultivation location in the middle of the North Atlantic Ocean is therefore different from cultivation taken place in e.g. France, Denmark, and Norway where the physical conditions like temperature and nutrients have larger seasonal fluctuations. In addition, these Faroese environmental conditions mean that *S. latissima* can be inoculated from September to March. The *S. latissima* can be harvested, in theory, all year round, but in practice is harvested from April to September where weather conditions allow working at sea. Furthermore, it has been shown possible to harvest *S. latissima* more than once and up to three years after deployment with two

annual harvests (multiple partial harvesting) [15]. The macroalgae are sold to the food and feed market, and consequently, the customers need to know the protein concentration and quality of the product they buy.

The present study aimed to evaluate the effect of the culture conditions: site (open ocean and in fjord), cultivation depth (1 and 9 meters below sea level; MBSL), and finally the harvest time (i.e. seasonal variation) with regard to the total nitrogen and protein concentration, and the amino acid composition of *S. latissima* cultivated commercially in the Faroe Islands. Protein concentration was determined by the sum of amino acids, and its nutritional value was evaluated based on the essential amino acid (EAA) composition and compared to reference patterns from WHO/FAO/UNU [12]. Moreover, the purpose was to establish specific N-to-protein conversion factors for Faroese *S. latissima* with regard to total amino acids and total nitrogen, in order to propose a general conversion factor for farmed Faroese *S. latissima*. Lastly, the possibility of having high quantity and quality of *S. latissima* was discussed in relation to a future macroalgal biorefinery.

2. Method and Materials

2.1 Cultivation and site description

The brown kelp species *Saccharina latissima* was cultivated by the company Ocean Rainforest Sp/F in the fjord Funningsfjørdur at the Faroe Islands (**Fig. 1**). The macroalgae were cultivated at two sites: the outer part and the central part of the fjord. The outer part of the fjord was termed the "exposed site" having occasional significant wave heights of 3-6m, was exposed to currents of 15–25 cm $\cdot$ s^{-1}, and a water depth of 50-70 meters [31,32], and the central site in the fjord was termed the "moderate exposed site" having occasional wave heights up to 3 meters, was exposed to similar or lower water current (no data exist), and a water depth of 20-30 meters [31]. The North Atlantic Current, which originates from the warm Gulf Stream, brings warm water to the area, providing a relatively stable water temperature ranging from 7 to 11°C during the year [27,28]. The salinity is stable at 35.0-35.2 [28]. The nitrate concentration is 8-12 µM during winter and spring and in most years starts to decrease in May during the spring bloom, and it

decreases more in the shallow waters than offshore. There is a large interannual variability in the timing of the decrease in nitrate concentrations as well as in the minimum level of nitrate concentrations during summer [30].

The macroalgae were cultivated on the MacroAlgal Cultivation Rigs (MACR) occupying a surface area of approximately 1 ha and carrying 2,500 meters of growth line each (**Fig. 2**; A). One MACR has a 500-meter-long horizontal fix line submerged 10 meters below sea level (MBSL) and 10-meter-long vertical growth lines attached on the fix line for every second meter; thus, carrying macroalgae growing from the sea surface and 10 meters down (**Fig. 2**; B & C). The MACR installation has proven to be survivable in the open ocean conditions since 2010 enabling economical feasible open ocean macroalgal cultivation [15]. In total, 10 km of growth line were seeded with *S. latissima* and deployed manually in November 2014.

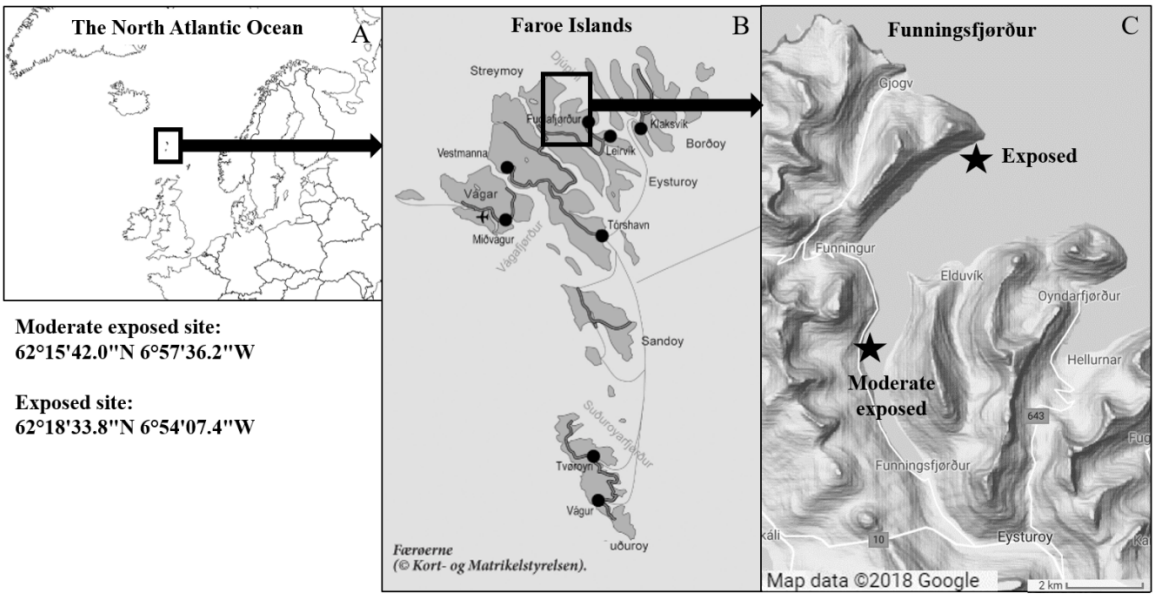

Fig. 1. Maps showing the North Atlantic Ocean (A, ©Wikimedia Commons, the free media repository), the Faroe Islands (B, © Kort- og Martrikelstyrelsen), and the fjord Funningsfjørður where the cultivation was done (C, © Google); the exposed and moderate exposed sites are marked with stars in the map.

Fig. 2. Two MacroAlgal Cultivation Rigs deployed next to each other under open ocean conditions at the mouth of Funningsfjørdur (A), the view of the 10-meter-long vertical growth lines seen from sea surface and down water column (B), and a growth line on the vessel held by the crane and ready for biomass sampling (C). All photos were taken by Ocean Rainforest Sp/F.

2.2 The sampling of *Saccharina latissima*

From the cultivated macroalgal biomass, 65 samples were collected during a 19 months period from deployment in November 2014 until May 2016 (**Table 1**). Not all months were sampled due to insufficient biomass or weather conditions. Samples were collected at the two sites "exposed" and "moderate exposed", and at two depths below sea level to compare the potential variation in biochemical

composition along a vertical growth line. The samples were cut either at the top of the line right under sea level (1-2 meters below sea level; MBSL) or at the lowest meter on the growth line (9-10 MBSL). The samples were termed “top” and “lower”, respectively.

Table 1. Sample overview; 65 samples of *Saccharina latissima* were collected from the cultivation sites in Funningsfjørdur (exposed and moderate exposed) and at two cultivation depths (top and lower). Not all months were sampled, as biomass could be insufficient, or weather conditions made sampling impossible. The numbers in the table represent biological replicates. The samples were all analysed for nitrogen concentration (N) and 35 of the samples were analysed for amino acids (AA) composition. The sum of AA was used to calculate total protein and nitrogen-to-protein factor.

	Nov-14	Mar-15			Apr-15	May-15			Jun-15			Jul-15			Aug-15			Sep-15	Oct-15			Nov-15	Dec-15	Jan-16			Feb-16	Mar-16			Apr-16			May-16			Total
	Deployed	E Top	E Lower	M Top	-	E Top	E Lower	M Top	E Top	E Lower	M Top	E Top	E Lower	M Top	E Top	E Lower	M Top	-	E Top	E Lower	M Top	-	-	E Top	E Lower	M Top	-	E Top	E Lower	M Top	E Top	E Lower	M Top	E Top	E Lower	M Top	
N		**2**	**-**	**-**		**3**	**-**	**3**	**3**	**-**	**3**	**3**	**3**	**3**	**3**	**-**	**3**		**3**	**3**	**-**			**3**	**3**	**3**		**3**	**2**	**3**	**3**	**3**	**2**	**-**	**-**	**3**	**65**
Protein		**-**	**-**	**-**		**3**	**-**	**3**	**2**	**-**	**3**	**-**	**3**	**-**	**3**	**-**	**3**		**3**	**-**	**-**			**3**	**3**	**2**		**-**	**-**	**3**	**3**	**2**	**2**	**-**	**-**	**-**	**35**

All samples were manually cut, leaving the holdfast, stem, and 5-10 cm of the blade for regrowth. Biomass from one meter of growth line was collected, so each sample had a weight of approximately 1.5 kg wet weight (ww) of several individuals and was collected from three replicate lines (n=3), excess seawater was drips off before stored in plastic bags. If epiphytes were present (always minor <5%) these were not removed in order to reflect normal harvest and product quality.

After sampling, replicates were ground for homogenising the sample using a SIRMAN fast chopper C4 VV and hereafter stored at -20 °C for maximum 12 months. All samples were freeze-dried followed by 15 seconds homogenizing (<5 mm size) with a Knifetec 1095 Sample Mill (Foss Tecator). The homogenized replicates were hereafter stored at -40 °C in plastic bags until biochemical analyses.

2.3 Dry matter and ash determination

Dry matter was determined for each sample by vaporizing water at 102-105 °C >20 hours until stable weight. Ash content was determined by incineration in a muffle furnace at 550 °C for 6 h.

2.4 Total nitrogen concentration using Dumas combustion

The total nitrogen concentration was determined by a nitrogen combustion method, the Dumas method, from approximately 0.5 g accurately weighed freeze dried and homogenized sample. The samples were analysed using the fully automated instrument 'rapid MAX exceed' produced by Elementar Analysensysteme GmbH with two analytical replicates (n=2) [33].

2.5 Amino acid profile, protein and protein quantity and quality

Samples of 30 mg dw were hydrolysed at 110 °C for 1 hour in 1 mL 6 M HCl in a microwave (Microwave 3000 SOLV, Anton Paar). Afterwards, derivatization followed using a Phenomenex EZ:faast amino acid analysis kit according to the user manual (Phenomenex). The amino acid composition was determined by liquid chromatography using mass spectrometry (Agilent 1100 Series, LC/MSD Trap) with an EZ:faast 4u AAA-MS column (250 x 3.0 mm, Phenomenex).

Determination of the total macroalgal protein concentration was calculated by summing up the total amino acids (TAA) in moles as recommended by Angell et al. [34] minus the water mass (18 g H_2O/mol amino acid) that was integrated during the disruption of the peptide bonds [23].

To calculate the essential amino acid (EAA) ratio the total EAA was divided by TAA. To evaluate the protein quality, the EAA score was determined following the procedure described by FAO [35] based on the amino acids requirement patterns for children age 3-10 from WHO/FAO/UNU[12], but not considering the digestibility.

2.6 Calculation of Nitrogen-to-protein conversion factors

Nitrogen-to-protein (N-to-protein) factors were determined for each sample by the ratio of total amino acid (TAA) residues minus water mass to the total nitrogen concentration (TN) of the sample [23].

Equation 1: N-to-protein factor = TAA/TN.

2.7 Statistical analyses

All data are expressed as mean ± standard deviation. PRIMER+ with PERMANOVA add-on package was used as statistical software. The nitrogen and protein concentrations, the N-to-protein factors,

and the total amino acid concentration were tested for homogeneity of variance using PERMDISP. Afterwards, the data were analysed by permutational analysis of variance (PERMANOVA) using Euclidian distances. A three-way PERMANOVA test of the interaction of the three factors; *depths* × *sites* × *seasons* was not valid because of missing data point and instead a two-way PERMANOVA was applied testing *depths* × *seasons* and *sites* × *seasons*. Whenever a significant difference between sample means or interaction of factors was revealed by PERMANOVA, a pairwise comparison among levels of factors was performed to compare the influence from *sites*, *seasons*, and *depths* on the compositions. In one case (total amino acids) a transformation of data was performed to achieve homogeneity of variance. Means were considered significantly different when levels of $p<0.05$ were obtained.

This was followed by multidimensional scaling (MDS) plot having one point for each sample. The points that were similar in composition were closer to each other and opposite was the distance between points larger if samples had more unlike composition (standardized samples by maximum resemblance, D1 Euclidian distance; 2-D stress, 0.17).

The results were finally analysed with a SIMPER analysis (based also on Euclidian distances) to identify those amino acid species that contributed most to the observed differences among time. Prior to this multivariate analysis of variance (PERMANOVA) all amino acid values were standardized and thereby expressed relative to the highest value in each dataset. The PERMANOVA analyses therefore only changed in composition, not in the total amount of amino acids.

3. Results

The Faroese cultivated *Saccharina latissima* samples were analysed for total nitrogen (TN) concentration and total amino acid (TAA) concentration (to express the protein concentration).

3.1 Depth, site and seasonal variation in the nitrogen concentration

The TN concentration ranged from 1.8±0.0% of dw (March 2015) to 2.5±0.5% of dw (May 2015) with an average of 2.2±0.3 % of dw for all samples (n=65; **Fig. 3**). The two-way PERMANOVA, testing

the effect of *sites* and *seasons,* revealed a significant seasonal variation in the TN concentration ($p<0.05$), whereas the site did not have a significant effect ($p=0.48$) nor did their interaction ($p=0.93$). However, the effect of *depth* and *season* for the TN concentration revealed a significant interaction of factors (PERMANOVA, $p<0.05$). Pairwise comparison showed that the difference in TN concentration between the top and lower samples was only significant in March 2016 ($p<0.05$; **Fig. 3**). The TN concentration was therefore not influenced by cultivation site and minor influence from different cultivation depths but influenced by a significant seasonal variation (**Fig. 3**).

3.2 Depth, site and seasonal variation in the protein concentration

The protein concentration (calculated by the total amino acids) varied from 2.9±0.3% of dw (June 2015) to 5.9±0.7% of dw (April 2016) with an average of 4.3±0.9% of dw of all samples (n=35; **Fig. 4**). The two-way PERMANOVA, testing the effect of *sites* and *seasons*, and *depths* and *seasons* for the protein concentration, revealed that there was no significant difference in the protein concentration between months ($p=0.06$), sites ($p=0.77$), or the interaction of these two factors ($p=0.17$). Moreover, there was no significant difference in the protein concentration between months ($p=0.43$), depths ($p=0.96$), or the interaction of these two factors ($p=0.52$). Consequently, there was no depth, site or seasonal variation in protein concentration for the cultivated *S. latissima.*

3.3 Specific nitrogen-to-protein factors

The N-to-protein conversion factor was calculated for the months analysed for both total protein and nitrogen concentration, and the average of all samples was 2.0±0.4 with the lowest factor (1.2) obtained in June 2015 and the highest factor (2.7) in April 2016 (**Fig. 5**). There was no significant difference in the N-to-protein concentration between months ($p=0.11$), depths ($p=0.89$), sites ($p=0.55$), or the interaction of these factors.

3.4 Amino acid profile and quality

The amino acid composition of the cultivated *S. latissima* is presented in **Table 2**. The highest EAA score was found in March 2016 at the moderately exposed site (106.4±11.2%) with lysine as the first

limiting amino acid. The lowest EAA score was seen in July 2015 at the exposed site (51.3±2.8%) with histidine as the first limiting amino acid. The main limiting amino acid was histidine (10 out of 13 cases including both sites) but in three individual cases valine, isoleucine and lysine were the limiting amino acids. The essential amino acid to total amino acid ratio was calculated and found to be lowest during winter (32.9-42.4%) and highest during spring and summer months (44.2-52.4%). The non-essential amino acids alanine, asparagine and glutamine had a large contribution to TAA in all months counting for approximately half of the concentration.

There was a significant seasonal variation in the amino acid composition (PERMANOVA, $p<0.05$) and a pairwise comparison showed that June 2015 and April 2016, October 2015 and January 2016, and March 2016 and April 2016 had a similar amino acid composition ($p=0.07–0.48$) and that all other months had a significantly different composition. No effect of cultivation sites or cultivation depths with regard to the amino acid composition was found (PERMANOVA, $p=0.17$ and $p=0.56$, respectively).

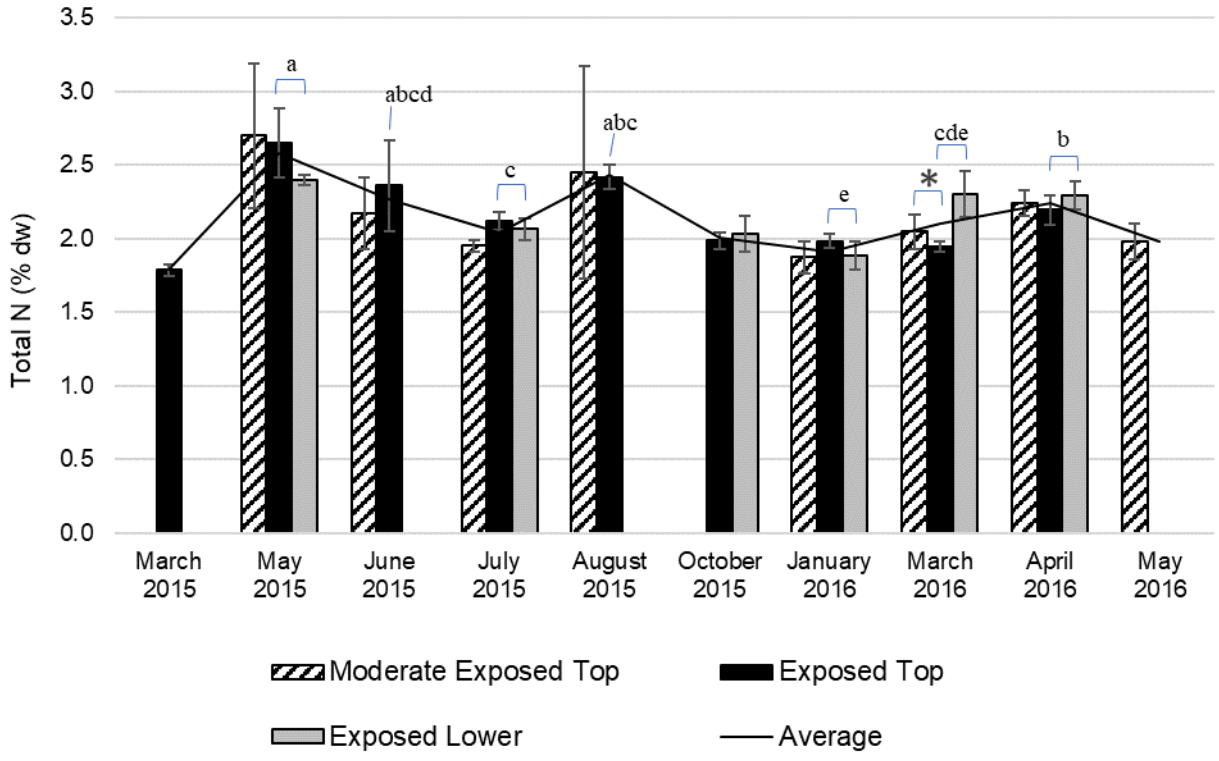

Fig. 3. Nitrogen concentration (% of dw) of Faroese cultivated *Saccharina latissima* at two sites (wave exposed, and moderate wave exposed) at two different depths (top: 0-2 MBSL and lower: 8-10 MBSL). The seeded lines were deployed in November 2014 and biomass was sampled from March 2015 until May 2016. Standard deviations are represented as bars (n=3), and the average concentration per month as a solid black line. A star (*) represents months with statistical different nitrogen concentrations between the top and lower samples. Different letters represent a significant difference between months (a,b,c,d,e) as a mean of both sides, as there was no statistical variation between sites.

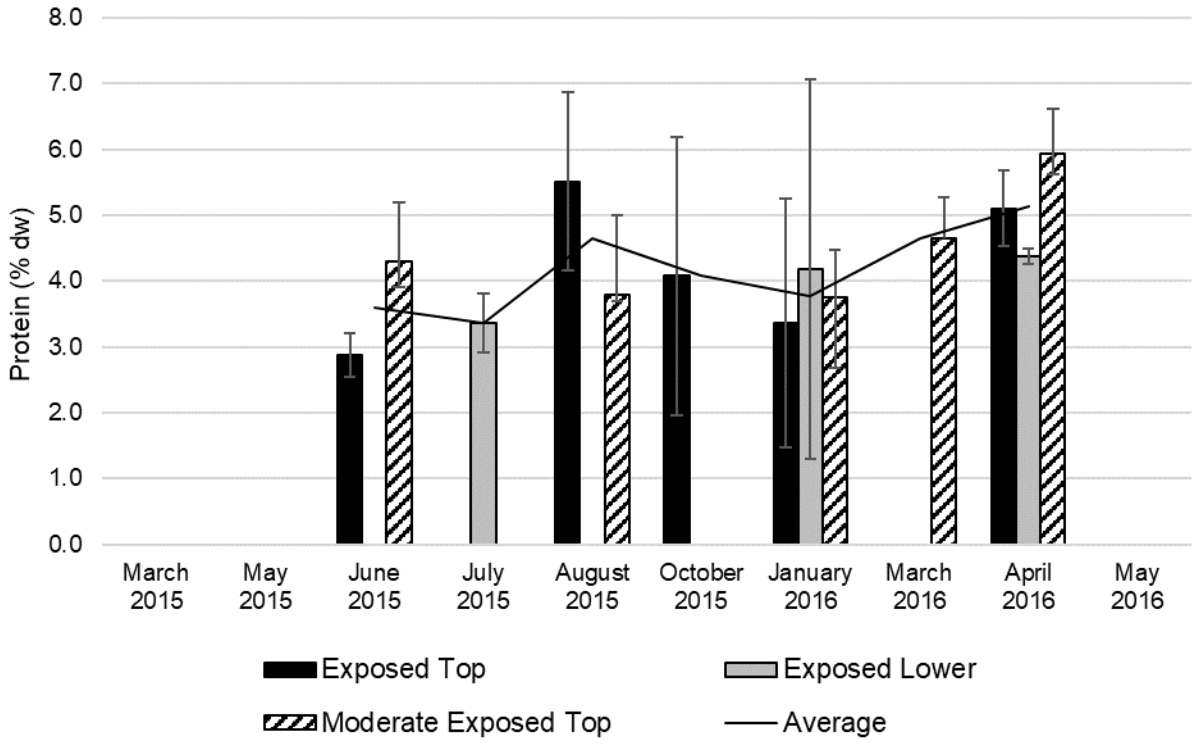

Fig. 4. The total sum of amino acids was used to determine total protein concentration (% of dw) of Faroese cultivated *Saccharina latissima* at two sites (wave exposed, and moderate wave exposed) at two different depths (top: 0-2 MBSL and lower: 8-10 MBSL). The seeded lines were deployed in November 2014. Samples were collected from March 2015 until May 2016; although, not all samples were analysed due to the high cost of amino acid analysis. Standard deviations are represented as bars (n=3), and the average concentration per month is shown as a solid line.

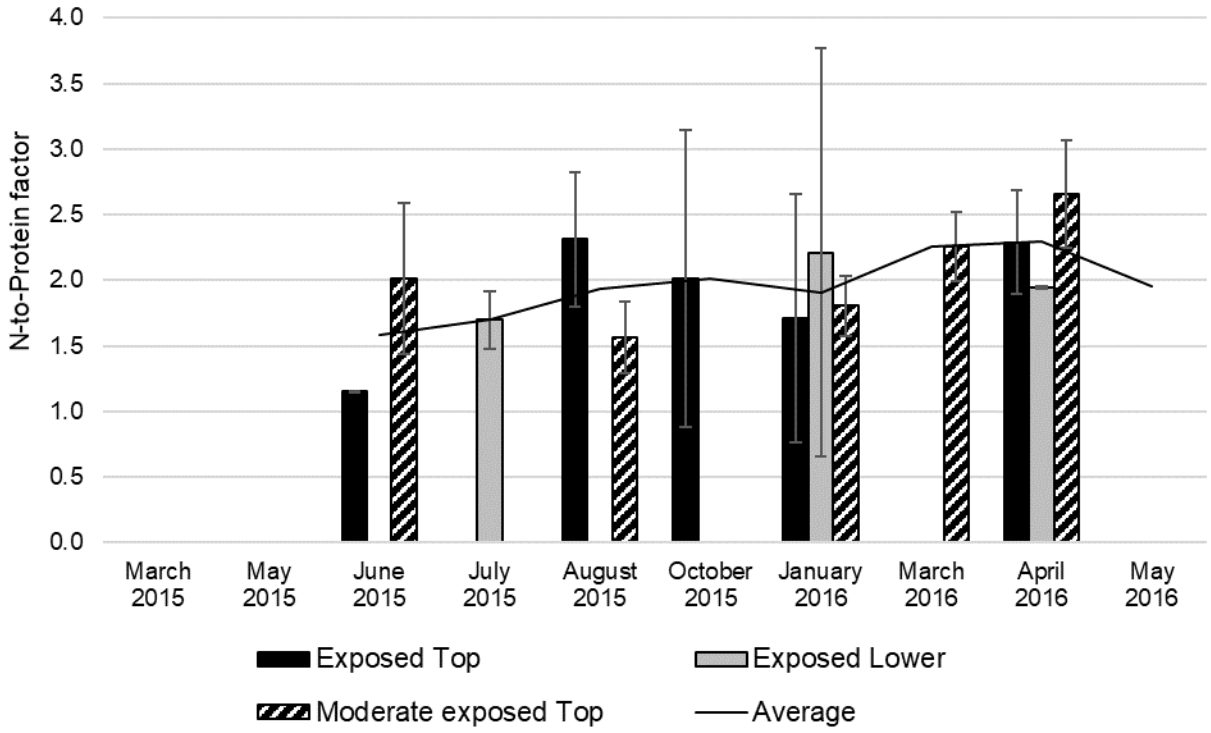

Fig. 5. The average calculated N-to-protein factors for Faroese cultivated *Saccharina latissima* at two sites (wave exposed, and moderate wave exposed) and at two different depths (top: 0-2 MBSL and lower: 8-10 MBSL). Samples were collected from November 2014 until May 2016. Standard deviations are represented as bars (n=3), and the average concentration per month is shown as a solid line.

Table 2. Amino acid composition in *Saccharina latissima* (mg amino acid g^{-1} protein), total essential amino acids (ΣEAA; mg g^{-1} protein), total amino acid concentration (TAA; mg g^{-1} protein), protein concentration (Protein; % of dw), essential amino acid ratio (EAA/AA), and an essential amino acid score (EAA score) cultivated at either exposed site or moderate exposed site at two different depths (lower and top) (June 2015-April 2016). Data expressed as mean±standard deviation (n=3). Essential amino acids in human are highlighted with grey boxes. n.d. = not detected.

Amino Acid	**June 15**		**July 15**	**August 15**		**October 15**	**January 16**			**March 16**	**April 16**		
[mg/g AA-protein]	**E, Top**	**M, Top**	**E, Top**	**M, Top**	**E, Top**	**E, Top**	**M, Top**	**E, Top**	**E, Lower**	**M, Top**	**M, Top**	**E, Top**	**E, Lower**
Lysine	58.8±16.5	68.1±11.2	116.3±15.0	54.2±7.6	69.5±17.3	61.8±3.4	72.6±22.7	41.6±7.3	50.5±3.2	51.1±5.4	81.0±13.8	89.5±14.8	62.0±4.7
Alanine	210.7±65.9	140.0±13.8	26.4±4.6	131.3±13.5	147.3±4.6	184.5±26.7	192.1	167.3±15.1	167.7±22.4	205.0±14.9	152.4±52.4	151.0±22.5	154.9±34.7
Arginine	26.2±2.6	30.4±4.4	51.6±12.6	95.4±30.1	77.0±40.8	44.1±21.0	29.4±3.2	46.1	66.8±13.6	107.2	35.1±6.0	33.4±7.8	35.1±8.0
Cysteine	3.4±0.2	5.9±1.3	3.8±1.4	3.9±0.9	3.6±0.9	4.1±2.1	5.6±1.7	5.6±2.5	4.0±2.4	2.9±+0.4	6.8	4.9±1.2	7.1±1.7
Leucine	70.7±6.6	75.7±7.6	61.6±4.7	90.6±8.8	87.3±10.5	70.6±9.9	49.7	84.9±4.3	78.±5.7	97.4±10.8	91.9±0.8	92.1±7.6	83.1±12.5
Methionine	44.3±3.8	38.3±6.6	30.5±10.9	39.1±5.0	35.3±9.6	30.9±6.3	21.2±3.4	43.8±9.4	39.8±15.6	39.6±3.5	30.5±3.6	28.6±1.7	32.5±5.6
Phenylalanine	42.2±5.0	50.7±8.4	73.4±7.1	71.4±12.4	62.2±17.0	43.7±10.6	37.2±9.4	60.1±5.5	56.1±6.9	68.6±6.8	55.6±4.2	53.5±7.0	49.9±11.6
Proline	58.0±1.8	59.4±4.1	27.2±4.1	63.9±7.2	64.3±6.3	50.2±1.4	42.0±12.2	61.2±3.2	52.6±3.3	68.7±7.6	56.2±6.1	61.4±5.0	69.7±2.6
Threonine	49.9±9.2	48.6±8.7	62.8±2.6	52.9±3.2	51.5±5.7	61.3±8.5	49.2±0.4	47.8±4.7	46.4±2.7	54.7±3.8	57.9±7.8	51.3±2.3	58.5±8.4
Tyrosine	29.4±4.1	33.0±3.1	50.0±1.8	36.2±2.4	33.6±3.4	28.5±4.9	25.7±8.0	27.4±1.8	29.6±7.4	34.7±3.6	29.0±3.5	32.9±2.8	32.0±3.9
Aspartic acid	148.7±21.3	150.9±15.2	251.4±21.2	171.5±2.7	156.1±20.1	168.8±47.1	173.9±48.2	145.7±13.6	142.9±12.5	146.1±2.2	134.6±10.3	141.1±26.1	147.3±16.5
Serine	62.3±6.6	56.2±4.5	64.4±5.0	68.8±3.8	59.8±6.9	71.5±16.0	59.0±3.1	50.9±9.4	53.9±6.6	61.2±5.0	49.5±7.6	54.5±5.3	60.3±1.5
4-hydroxyproline	3.3±0.7	11.8±13.0	4.2±0.7	5.1±0.7	4.3±0.2	6.1±0.9	8.1±0.1	6.4±2.5	6.8±0.9	3.8±0.2	5.0±0.9	5.1±0.6	7.3±1.3
Glutamic acid	162.1±3.2	178.5±17.4	216.2±34.0	144.0±19.8	155.9±19.1	276.4±52.7	332.9±60.3	223.2±10.2	232.7±27.3	136.7±11.0	146.2±0.4	136.4±25.3	147.7±2.5
Valine	55.4±5.7	76.5±40.4	20.5±1.1	44.3	56.2±1.2	53.3±9.6	35.3±17.3	56.5±11.3	77.1±10.2	70.3±13.8	83.1±7.0	80.1±8.7	80.3±2.5
Histidine	12.7±3.3	12.9±4.7	17.6±1.2	12.7±1.5	13.9±2.6	9.7±2.2	11.6±1.1	13.9±1.7	11.1±1.4	17.6±2.8	13.4±2.5	13.1±0.3	10.1±6.8
Isoleucine	41.9±6.0	37.8±3.4	27.2±7.1	30.7±5.7	34.4±6.9	38.8±8.7	17.4±2.5	34.2±1.9	32.7±7,4	33.2±6.5	52.3±18.0	44.3±4.4	36.6±2.8
Glycine	103.7±14.1	100.1±23.7	46.5±39.0	58.7±10.5	58.7±30.1	109.1±19.1	74.4±37.8	88.7±34.4	69.7±33.4	47.6±4.1	99.9±23.8	102.3±11.7	103.1±28.5
ΣEAA	405.4±53.6	441.5±42.0	459.9±23.1	402.6±31.2	444.0±34.3	385.7±64.4	319.9±19.8	410.1±9.2	396.2±42.0	467.2±48.1	494.7±52.5	485.5±25.6	445.0±53.9
TAA	914.3±42.8	966.5±19.2	1009.1±24.2	911.8±122.2	954.3±9.4	1054.1±211.4	972.7±57.3	968.3±19.3	955.4±21.5	920.3±19.0	943.6±39.3	935.0±12.9	960.6±23.0
AA-Protein (%)	2.9±0.3	4.3±0.9	3.4±0.4	3.8±1.2	5.5±1.3	4.1±2.1	3.8±0.7	3.4±1.9	4.2±2.9	4.6±0.6	5.9±0.7	5.1±0.6	4.4±0.1
EAA/TAA	44.2±3.8	45.6±3.7	45.6±2.0	44.4±3.4	46.5±3.3	36.8±3.0	32.9±0.1	42.4±1.8	41.4±0.04	50.8±4.9	52.4±3.4	51.9±3.3	46.3±4.5
EAA score (%)	**79.5±20.5**	**80.5±29.6**	**51.3±2.8**	**79.4±9.4**	**87.0±16.1**	**60.5±14.0**	**56.0±8.2**	**86.6±10.7**	**69.1±8.6**	**106.4±11.2**	**83.5±15.8**	**82.2±2.1**	**63.3±42.8**
Limiting EAA	Histidine	Histidine	Valine	Histidine	Histidine	Histidine	Isoleucine	Histidine	Histidine	Lysine	Histidine	Histidine	Histidine

Tryptophan was not detected.

3.5 Multidimensional scaling of the amino acid composition

A multidimensional scaling (MDS) plot of the amino acid composition in the cultivated *S. latissima* showed a grouping of the samples; one point for each sample was used and marked by month (**Fig. 6**). The points that are close were more similar in their composition such as samples from June 2015 and April 2016 (all samples within the circle are very similar), compared to points further apart (outside the circle). July 2015 samples are the ones that are most separated from the other months, which can be related to a much lower concentration of alanine and valine and relatively high level of lysine in comparison with the other months. Samples from winter (October 2015 and January 2016) tend to be high in glutamic acid and separated from the other months in the MDS plot.

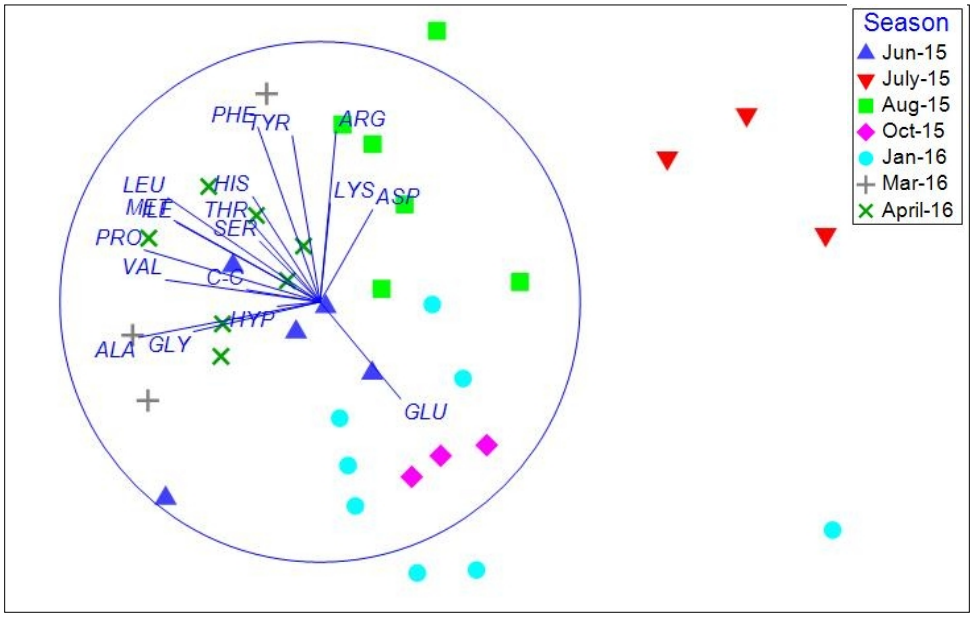

Fig. 6. A multidimensional scaling (MDS) plot, one point for each sample (standardized samples by total resemblance, D1 Euclidian distance; 2-D stress, 0.17), considering the similarities of the amino acid composition of the cultivated *S. latissima* (n=3-8). The closer a point is to another the more they have in common. The circle shows the main distribution of samples.

3.6 Similarity Percentages Analysis (SIMPER)

The amino acids that contributed to the observed differences in the composition among pairs of months were tested using a similarity analysis (SIMPER). The result was only relevant for pairs of months that differed significantly in the pairwise comparison. From the MDS plot the July, October 2015 and January

2016 months had the largest distance to a main distribution of the samples. The SIMPER test showed that alanine was the main reason why July 2015 was different from the others (counting for 32-39%). For October 2015 the main contributor to the changes was glutamic acid (counting for 43-66%). In January 2016 the main contributor to the changes was glutamine (counting for 48-61%).

4. Discussion

4.1 Influence of environmental factors on nitrogen and protein concentration

Growth, including the formation of proteins, is correlated with available light, nutrients (primarily nitrogen) and water motion [13,16,36]. Most algae, like *S. latissima*, can take up excess nitrogen and store it internally as nitrate or urea for the later utilization when nutrient availability in the water is low. In this study, no difference in the nitrogen concentration was found between depths (except in March 2016) or between sites, but there was a significant seasonal variation (section 3.1). The seasonal variation in nitrogen is, therefore, most likely related to the availability of nitrogen in the sea, internal nitrogen storage of the alga and the incorporation of nitrogen in building tissue. The average nitrogen concentration was 2.1±0.2% of dry weight (dw) in accordance with other studies using the same determination method [20,38].

The average protein concentration (calculated by the total amino acids, hereafter AA-protein) was 4.3±0.9% of dw, and no depth, site or seasonal variation of the protein concentration was present, though seasonal variation was close to being significant, having a *p*-value of 0.06. The AA-protein concentration showed large standard deviations, and this can explain the natural variation among individuals or that more biological replicates are needed to get a better understanding of the variation in protein between samples from different seasons, sites and depths. Despite the large standard deviation, the lack of seasonal variation of the AA-protein concentration could be a consequence of the stable physical environmental conditions seen in the Faroe Islands i.e. stable temperature and salinity [27].

Contrary to salinity and temperature, the irradiance and day length varied substantially through the year in the Faroe Islands [15], nevertheless, the incoming light at the two sites was close to equal as the

distance between sites is only six-kilo meters. The sites had therefore equal light, temperature, salinity, and current conditions, and the difference between the sites was the wave height and water column depth, and these differences were here found not to have any significance for the nitrogen and protein concentration.

Environmental factors may vary with depths [13], and because *S. latissima* was cultivated on 10-meter-long vertical growth lines, the depth could affect the nitrogen and protein concentration along the line and the variation with regard to depth was consequently examined. A significant depth variation in nitrogen concentration occurred only in March 2016. Here, the nitrogen concentration in the top sample was 2.0±0.0% dw and the lower sample was 2.3±0.2% dw. The difference could be explained by high competition for nutrients between the macroalgae growing in the top of the lines and phytoplankton as spring is the season where phytoplankton blooms often take off, and thus being nutrient limited for a period in March.

There was no statistical difference in AA-protein concentration between cultivation depth, which may indicate that periods of limited nitrogen levels are short and that *S. latissima* can use their internal storage of nitrate and urea.

4.2 The comparison of analytical methods for measuring nitrogen concentrations

All compounds (e.g. ash, polysaccharides, protein, lipids) have their share of the proximate composition of the macroalgal biomass. The concentration of the compounds is relative to one another, so if one compound or yield increases this reduces the concentration of other compounds. The below section compares the different methods applied in order to find the method that gives the protein result closest to the truth of the macroalgal biomass at a given time of year, taking the entire proximate composition into consideration. This is valuable for trading biomass as well as experiments that rely on the given amount of protein in the biomass for example feeding trials.

Nitrogen analysis be performed by methods such as the Kjeldahl method [38] or the Dumas method [39]. Kjeldahl is the international reference method for nitrogen determination, but the Dumas method has recently been developed to improve its accuracy and running time and can be fully automated. The Kjeldahl method is not measuring inorganic forms of nitrogen, such as nitrate and nitrite, as they might not be

sufficiently degraded by digestion. In contrast, all nitrogen sources, are measured by getting all N on gaseous form in the Dumas method [33]. This is important when determining nitrogen in biomass with high levels of inorganic forms of nitrogen. As the Dumas method determines all the nitrogen forms, slightly higher levels of nitrogen are usually found when using the Dumas method compared to the Kjeldahl method [22]. As protein concentration of brown macroalgae often has been estimated as crude protein by determining nitrogen concentration by analysis and then multiplying with an N-to-protein conversion factor (often 6.25), it can be concluded that the approach of analysis of nitrogen concentration could, in the end, have an influence on the crude protein results.

4.3 Comparison of protein concentration between studies

Reported protein content of seaweeds depends on the analytical method used for determination, which are not based on quantifying the same components. Consequently it becomes difficult to make a direct comparison between studies. Vilg et al. [40] used the colorimetric Lowry Protein Assay [41], other studies were based on quantifying nitrogen by either Dumas or Kjeldahl followed by the use of N-to-protein conversion factors of either 5.0 [36] or 6.25 [42] (hereafter called the crude protein). However, most studies used the same method as this study by summing up the amino acid residues (AA-protein) and subtracting the water molecules added during hydrolysis [9,17,21,24,37].

The AA-protein method is the only method where interfering substances do not affect the results; however, there is potential for improvement in regard to the hydrolysis method [43]. In the following section, protein content of *S. latissima* is compared between studies. Only studies that use the AA-protein determination method, as used in this study, will be addressed for accurate comparison.

Mols-Mortensen et al. [21] cultivated *S. latissima* at three sites with different exposures (sheltered, current exposed and wave exposed) in Faroese waters and characterized the growth and quality of the biomass and the available nutrients in the surroundings from March to August 2015. Therefore, very similar to our study. However, some variations in the performed work differ from the cultivation of this current study and the results by Mols-Mortensen et al. [21] can therefore not be directly applied to all Faroese cultivated *S. latissima*. The cultivation by Mols-Mortensen et al. [21] had two meters long cultivation lines

and 10 replicate lines. The site "wave exposed" had a max significant wave height of 2.2 meters [21], which is the same wave height of the "moderate exposed site" of this study. Finally, they did analyse one growing season, whereas our study investigated two growing seasons. Mols-Mortensen et al. [21] found a significant seasonal variation in AA-protein concentration over the year, opposite to this study, and they found no significant difference between sites, similar to our study. All samples from Mols-Mortensen et al. [21] (except July at the sheltered site) had higher AA-protein concentration than the *S. latissima* cultivated in Funningsfjørdur (~5-17% of dw), even though the determination method was the same.

Marinho et al. [9] collected bi-monthly samples from *S. latissima* (including epiphytes, when present) cultivated commercially at an integrated multi-trophic aquaculture (IMTA) site, and from a reference site in Denmark. Overall, there was no significant difference in biomass composition between the two sampling sites, like our study. However, seasonal variations in AA-protein and nitrogen were found, which was also the case for this study regarding nitrogen. The AA-protein concentration varied markedly reaching a maximum of 10.8% dw in November 2012 and a minimum of 1.3% dw in May 2013 in the study Marinho et al. [9]. The presence of epiphytes was found as a major issue in the Danish cultivation study and therefore the summer harvest was suggested to be used for feed instead of food.

Nielsen et al. [37] analysed AA-protein concentration at ten sites in the inner Danish waters using wild collected *S. latissima* and found an average AA-protein concentration of 3.1% of dw in August 2012. They concluded that depth did not have a significant influence on the AA-protein concentration, in line with our results. Instead, they concluded that sites with different salinity had a significant influence on the AA-protein concentration.

The AA-protein concentration found for cultivated *S. latissima* in Funningsfjørdur (2.9-5.9% dw) was in the lower end compared to concentrations found by other studies [9,21], although Nielsen et al. [37] had similar levels (1.1-7.5% of dw). The seasonal variation of AA-protein concentration from this study was not significant, though close (p=0.06), which are reverse results found in other studies were seasonal variation was present. Several literature reviews found *S. latissima* to have the highest AA-protein concentration in winter months or early spring [2,3,44], which was also the conclusion by Mols-Mortensen

et al. [21]. Contradictory, Marinho et al. [9] had the highest AA-protein concentration in autumn for *S. latissima* cultivated in the inner Danish waters.

4.4 Protein determination with nitrogen-to-protein conversion factors

The average N-to-protein conversion factor found for *S. latissima* cultivated in Funningsfjørdur was 2.0±0.4, and therefore much lower than the widely used 6.25 or 5.0. Other studies found conversion factors for *S. latissima* to be 3.9 [37], 3.7±1.3 [45] and 0.9-4.5 (*manuscript in prep.,* Marinho & Holdt). Authors within both macroalgae, meat, fish, and plant science determine crude protein by using conversion factor of e.g. 6.25; although, it has been acknowledged that plant or macroalgal protein differ in terms of nitrogen concentration thus this factor is overestimating the concentration [22]. Therefore, several studies have calculated species-specific N-to-protein conversion factors for macroalgae to avoid overestimating. Angell et al. [34] suggested a prioritized list of methods for protein determination, which depended on the knowledge of species-specific conversion factors and the availability of analytical methods. They suggest the total amino acids concentration (TAA) to be the most precise, which is also supported by FAO [8] and Mæhre et al. [43]. However, if this analysis is not financially or technically available the species-specific N-to-protein conversion factor should be used. A third option recommended is to use a macroalgal specific conversion factor of 5.0.

If the general macroalgal conversion factor of 5.0 on nitrogen concentration was applied on the results of the present study the crude protein concentration would in average be 10.8±0.9% of dw compared to 4.3±0.9% of dw when summing TAA. A comparable study applying the conversion factor 5.0 was Bruhn et al. [36], which was based on nitrogen concentrations determined by the Kjeldahl method. They found a crude protein concentration of *S. latissima* from the inner Danish waters of 16-17% of dw, which is above the crude protein levels found in Funningsfjørdur, but a comparison is not possible due to the determination method.

These various protein determination methods will overall lead to several consequences. Commercially consequences would be to suggest potential harvest times and places for highest protein concentration as these are not sufficiently expressed if the nitrogen concentration is multiplied by a single

factor on all results. Therefore, it is recommended to quantify the total amino acids, which will also indicate the AA-protein quality. The economic value of food and feed is often established based on the protein quantity and quality. By applying a single N-to-protein conversion factor (e.g. 5.0 and 6.25) this will lead to an over- or underestimation of the actual value of the macroalgal proteins. Hence, the species and season-specific N-to-protein conversion factors should be used with absolute care and with the knowledge that the results from the conversion would only represent an estimation of crude protein concentration. Adding to the species and season-specific conversion factor, a site and depth-specific N-to-protein conversion factor can be used. However, as a result of this study, this is not significantly influencing the protein concentration, and these multiple options can lead to a problem when comparing protein concentration between studies, but also increases the complexity of determining the protein concentration. For all these reasons we recommend the method of summing total amino acids for total protein estimation of macroalgae.

4.5 Amino acid profile and protein quality

All analysed amino acids were found in the samples. The amino acids analysed represented both protein-derived amino acids and free amino acids. The presence of free amino acids contributes to an overestimation of the total protein content, though typically counting for less than 10% [24]. However, the procedure is widely accepted, since in acid hydrolysis some amino acids are partially or totally destroyed e.g. tryptophan [46], and the total amino acids will in this way be acceptable as the free amino acids are adding and the destroyed amino acids are reducing [24].

Aspartic acid and glutamic acid were the dominant amino acids obtaining 30.2-52.1% of the total amino acids (TAA). These results are in agreement with those reported for other brown macroalgae where these two amino acids accounted for 22-49% of TAA [3,9].

The EAA/TAA ratio, which is a way to estimate the quality of the protein as food, was in our study found to be in the range between 33% and 52% with the lowest values found in January 2016 and highest in April 2016. From the studies of *S. latissima* in the Danish inner waters, the ratio was reported to be between 26% and 30% for wild populations [37] and the ratio for cultivated *S. latissima* was reported to be between 21% and 42% [9].

The quality of the protein was also described using the total EAA score and levels above 100% meant that more than the required human intake was met, thus high-quality protein. The EAA score was lowest in July (51.3±2.8%) and peaked in March 2016 (106.4±11.2%), representing enough essential amino acids compared to the reference patterns from WHO/FAO/UNU [12]. Mols-Mortensen et al. [21] found highest EAA score in May 2015 (93.7%) on the Faroese cultivated *S. latissima* and Marinho et al. [9] found highest EAA score in November 2013 (68.9%) from *S. latissima* cultivated in Denmark.

In this study, histidine was the main limiting amino acid with three exceptions. Histidine was also reported to be the limiting amino acid in the study from both Marinho et al. [9] and Mols-Mortensen et al. [21]. Furthermore, the EAA concentration of the *S. latissima* cultivated in Funningsfjørdur in all sampling periods was above the requirement pattern (305 mg g^{-1} protein) by WHO/FAO/UNU [12].

It is important to comment that nutritional value mainly is defined by both amino acid composition and digestibility [11,12]. In order to fully evaluate the biological value of protein from *S. latissima*, in vivo protein digestibility trials must be carried out. Since the protein concentration of Faroese *S. latissima* is in the very low end of macroalgae and other food sources its potential solely as a protein source is very limited. Although the protein concentration is low, it is interesting to investigate if *S. latissima* can be utilized in the food and feed industry as it is an underexploited resource.

5. Conclusion

Saccharina latissima cultivated in the Faroe Islands had a significant seasonal variation of nitrogen concentration, though the AA-protein concentration did not show significant seasonal variation (p=0.06). There was no difference found between the moderate exposed and exposed cultivation sites and the difference between macroalgae grown in the top meters was not (except one month) significantly different in terms of nitrogen and AA-protein concentration from the macroalgae grown at 9 meters below sea level. This means that the companies that cultivate *S. latissima* in Funningsfjørdur do not need to distinguish

according to the present findings between cultivation sites, depths or seasons when informing about protein concentration, which is contrary to cultivation in other geographical areas like e.g. the inner Danish waters.

Cultivated Faroese *S. latissima* had a high proportion of essential amino acids (EAA), a high EAA/TAA ratio, and an EAA score above 100% in March 2016. The Faroese cultivated *S. latissima* can, therefore, be recommended as a future source for high-quality food and feed, optimally harvested in March but also when harvested in other months as the EAA score in was generally high.

The average N-to-protein conversion factor found in this study was 1.96 and thus about two thirds less than the widely used conversion factor of 6.25 for crude protein. Consequently, specific N-to-protein factors are not recommended for use for total protein determination, since it could lead to an over- (or possibly under) estimation. Therefore, it can be concluded that determining protein concentration should preferably be made by the use of quantitative amino acid analysis (AA-protein).

Moreover, this present study stresses the need for further investigation of sustainable and economical-feasible methods for protein extraction to utilize the high quality but low quantity protein source in offshore cultivated *S. latissima*.

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Conflict of Interest

The authors declare no conflict of interest.

Informed Consent, Human/Animal Rights

No conflicts, informed consent, human or animal rights applicable.

Declaration of authors contribution

All persons designated as authors qualify for authorship and are listed as authors. Each author has participated sufficiently in the work to take public responsibility for appropriate parts of the content. All authors have made substantial contributions to the conception and design of the study, acquisition of data, and/or analysis and interpretation of data. All authors have drafted the article and/or revising it critically for important intellectual content. And all authors have given their final approval of the version to be submitted.

Appendix A. Supplementary data

Supplementary data to this article can be found online at XXXX.

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PAPER IV

Seasonal, site and depth variation and chemical risk evaluation of total iodine in offshore commercially cultivated Faroese *Saccharina latissima*, *Alaria esculenta* and *Laminaria digitata*.

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Seasonal, site and depth variation and chemical risk evaluation of total iodine in offshore commercially cultivated Faroese *Saccharina latissima, Alaria esculenta* and *Laminaria digitata*

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Abstract

The demand for sustainable cultivated macroalgal biomass as a sustainable food and feed ingredient is increasing. Macroalgae may contain high levels of iodine typically in the g/kg dw range. When macroalgae are used in food and feed products, it can be both beneficial as a good dietary iodine source, or a potential concern, as even small amounts of biomass can lead to too high iodine exposure. Consequently, increased knowledge and awareness on this topic is crucial for safety of macroalgal food and feed ingredients and ultimately for the European macroalgal industry to grow. Commercial macroalgal biomass was cultivated offshore in the Faroe Islands and was sold to the food and feed market. The seasonal variation of total iodine content of cultivated *Saccharina latissima*, *Alaria esculenta* and the self-seeded *Laminaria digitata* was analysed to determine optimal harvest time, and to estimate the exposure to iodine by consumption of seaweeds. A significant variation in total iodine content was found among the three seaweed species. *L. digitata* had the highest iodine content (average ± standard deviation) of 5,400±1,400 mg/kg dw, followed by *S. latissima* with 4,000±1,700 mg/kg dw which also had a significant seasonal variation with low iodine content during spring and summer, and *A. esculenta* had the lowest levels of iodine (250±140 mg/kg dw). No significant variation in iodine was found when cultivated under different site conditions, different depths or when deployed in two different years. The chemical risk assessment of an excessive iodine intake suggests a safe intake level of 0.7-2.1 g dw/day for *S. latissima*, 0.7-1.3 g dw/day for *L. digitata* and 12-35 g dw/day for *A. esculenta* based on the seasonal variation of iodine, the established tolerable upper level of consumption and a 17% bioavailability of the iodine.

Keywords

Chemical composition, kelp, macroalgae, seaweed, risk assessment, food, feed.

1 Introduction

Macroalgae, commonly known as seaweed, have played an increasing role in human diet, health, and medical purposes especially in Asian countries for more than thousand years (Levine 2016). In Asia, and particularly in Japan, macroalgae have been and still are a staple food commodity. The Japanese are considered one of the longest-living people in the world, which has been linked to their unusual high intake of iodine (1-3 mg/day) with macroalgae as the primary contributor (Zava and Zava 2011).

In western countries, a limited number of the population is consuming macroalgae on a regular basis (Fleurence et al. 2012). However, the increasing utilization of macroalgae for example due to the popularity of sushi (Edwards et al. 2012) and the increased focus on innovative product development has raised the appreciation for novel applications of this organism (Cottier-Cook 2016; Buschmann et al. 2017; Ferdouse et al. 2017). Macroalgae have been experiencing a frameshift from the smelly stuff on the beach to a useful raw material serving as a source of various compounds with a variety of applications (O'Sullivan et al. 2010; Holdt and Kraan 2011; Lordan et al. 2011; Fleurence and Levine 2016).

Generally, large consumption of macroalgae has been linked to decreased rates of many "Western lifestyle" diseases such as cancer and cardiovascular diseases (e.g. O'Sullivan et al. 2010; Holdt and Kraan 2011; Lordan et al. 2011). Macroalgae are a great source of bioactive compounds. Bioactive compounds including polysaccharides, proteins, lipids and polyphenols derived from macroalgae have demonstrated several beneficial biological activities (Val et al. 2001; Yuan and Walsh 2006; Chandini et al. 2008; Kang et al. 2008; Pushpamali et al. 2008; Sinha et al. 2010).

Furthermore, macroalgae absorb high amounts of a diverse group of minerals and trace elements from the marine environment. The accumulation of calcium, iron, and copper is greater in macroalgae than in terrestrial sources, and macroalgae are a good source of trace elements such as magnesium, copper, iron, iodine, and zinc (MacArtain et al. 2007). However, accumulation also occurs of elements that are toxic to humans such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) (Desideri et al. 2016), and regular consumption of even small amounts of macroalgae may lead to adverse health effects.

Iodine is an essential mineral for humans; though, too high iodine intake can lead to adverse effects. Iodine is accumulated to a very high degree in some brown macroalgae, and consumption of these macroalgae may, therefore, lead to a positive or negative effect on human health depending on the iodine quantities ingested.

Globally, approximately 31% of the world population has insufficient iodine intake (WHO et al. 2007). In Europe, about 50% of the population is mildly iodine deficient (Zimmermann 2009). Consequently, consumption of macroalgae could help to treat this iodine deficiency. On the other hand, consumption of some macroalgae with high iodine levels could lead to intakes of iodine that pose health risks to humans. That is why the assessment of chemical safety of macroalgae has attained increasing focus in recent years (e.g. Lüning and Mortensen 2015; Nitschke and Stengel 2016).

In the present study, the concentration of iodine in three brown macroalgae *Saccharina latissima*, *Alaria esculenta* and *Laminaria digitata* samples was quantified. The macroalgae were harvested from two different cultivation sites in the Faroe Islands, at two different cultivation depths, and monthly in two consecutive years (2015-2016). The relation of age and iodine content was investigated for the one-year-old and two-year-old algae, and the difference between years was also analysed. It is expected that the results obtained can contribute to a better understanding of the seasonal

variation of the iodine content in cultivated *S. latissima*, *A. esculenta*, and *L. digitata*, and contribute to an improved harvest strategy. The study will finally recommend a daily intake of the macroalgal species studied based on a chemical risk assessment including dietary exposure and legislation.

2 Method and Materials

2.1 Cultivation method and site descriptions

The brown kelp species *Saccharina latissima* and *Alaria esculenta* were cultivated in Funningsfjørdur at the Faroe Islands (Figure 1). The macroalgae were cultivated at two sites: the outer part and the central part of the fjord. The outer part of the fjord was termed the "exposed site" (water depth 50-70 meters) having occasional significant wave heights of up to 6 meters and exposed to currents of 15–25 cm · s^{-1} (Bruntse et al. 1999; Norði and Patursson 2012). The central site in the fjord was termed the "moderately exposed site" (water depth 20-30 meters) having occasional wave heights up to 3 meters and was exposed to similar or lower water current (no data exist) (Bruntse et al. 1999). The North Atlantic Current, which originates from the warm Gulf Stream, brings warm water to the area, providing a relatively stable water temperature ranging from 7 to 11°C all the year round (Bruntse; Larsen et al. 2008) and a stable salinity at 35.0-35.2 (Bruntse et al. 1999).

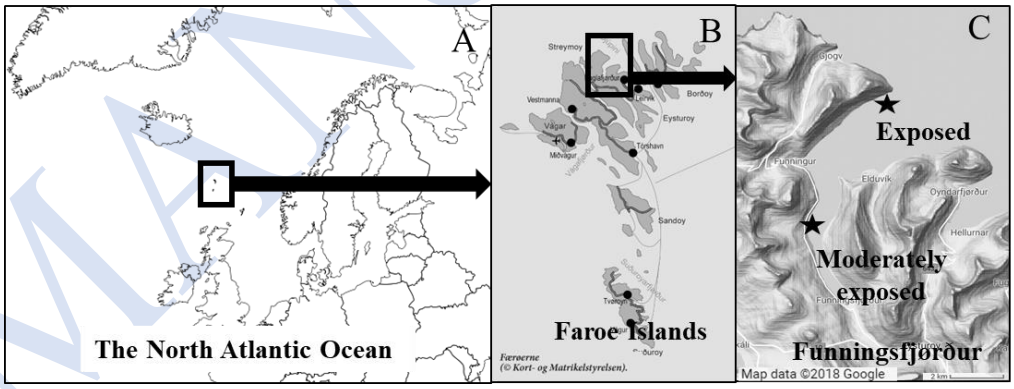

Figure 1. Maps showing the North Atlantic Ocean (A, ©Wikimedia Commons, the free media repository), the Faroe Islands (B, © Kort- og Martrikelstyrelsen), and the fjord Funningsfjørður, where the cultivation was done (C, © Google); the exposed (62°18'33.8"N 6°54'07.4"W) and moderately exposed sites (62°15'42.0"N 6°57'36.2"W) are marked with stars in the map.

The macroalgae were cultivated using a Macroalgal Cultivation Rig (MACR) developed by the company Ocean Rainforest Sp/F, and this rig was designed for upscaled/commercial macroalgal cultivation and to withstand the weather conditions of the North Atlantic Ocean (Bak et al. 2018). The design consisted of a 500-m long fix-line suspended horizontally at 10 meters below sea level. Two main surface floats were connected to the fix-line and the mooring system consisted of four anchors. The rig had approximately 250 growth lines of 10-m length attached to the fix-line with a float fixed at the opposite end, stretching the lines in a vertical position (Bak et al. 2018).

A total of 10 km seeded line was deployed in November 2014 attached on two MACR's in the "exposed site" and two MACR's in the "moderately exposed site". *A. esculenta* was only deployed in the "exposed site". The seeding material was produced by the company Hortimare BV, the Netherlands, using a standard procedure for kelp sporulation (Edwards and Watson 2011). Fertile *S. latissima* and *A. esculenta* were collected from wild populations in Funningsfjørður in January 2014. The spores were released to sterile and filtrated seawater and nursed till enough biomass was reached (cultivated in vitro for approximately 10 months). After an induction period, the gametophytes developed into juvenile sporophytes (size <1 mm). The juvenile sporophytes were seeded on 2-mm lines using a binder-mixture produced by Devan Chemicals N.V. with the product code DG518. The seed lines were twined around coils and the juvenile sporophytes were cultivated in hatchery tanks for a three-week period before deployment. The seed lines were twined around the 10-m long growth line (specified in Bak et al. 2018).

The macroalgal biomass was harvested using a multiple partial harvest method. The method only cuts off the blades and leaves the hold-fast, stem and 5–15 cm of the blade on the line to ensure the preservation of the meristematic zone to allow regrowth. The macroalgal samples for iodine

analysis were harvested using the same method. The lines were kept in the sea for two years. In the second year of cultivation, a number of self-seeded *L. digitata* were growing on the lines. These specimens were also used for sampling as this species has a commercial interest as a self-seeded crop.

In December 2015, two more MACR's were deployed seeded with *S. latissima* and these lines were used to analyse the difference in iodine content between first- and second-year algae.

2.2 Sample collection and preparation

Saccharina latissima (n = 65), *Alaria esculenta* (n = 30) and *Laminaria digitata* samples (n = 18) were analysed for total iodine. The samples were collected monthly in the period from May 2015 to November 2016 at the two cultivation sites "exposed" and "moderately exposed" and at two different depths "0-2 MBSL" and "8-10 MBSL". Samples were collected from three different lines. If epiphytes and epifauna were present these were not removed. Approximately 1-1.5 kg wet weight biomass per sample was cut off and stored in closed plastic bags.

In the laboratory, samples were grinded into pieces of 1-2 cm^2 (SIRMAN fast chopper C4 VV). The samples were stored frozen (-20°C) until transportation to the laboratory at DTU Food, Denmark for subsequent iodine analysis. During transportation, samples were stored on ice. The samples were freeze-dried (Martin Christ Gefriertrocknungsanlagen GmbH, Christ E278, type 100800) and homogenized into a fine-grade powder using a dry-food grinder for approximately 10-15 s after which the homogenates were stored at -20°C. Determination of the dry matter content of the freeze-dried biomass was conducted by drying (105°C; 24 h) to enable reporting of results in mg iodine per kg dry weight (dw).

2.3 Extraction and analysis of total iodine content

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was applied to determine total iodine content in the macroalgae samples. Determination of total iodine followed the principles of the standard (EN 15111:2007) issued by the European Committee for Standardization (CEN) in 2007. Water was ultra-purified (<18 MΩ cm) using a Milli-Q-Integral system (Merck, Germany). Tetra-methyl-ammonium-hydroxide (25 % w/w TMAH, Alfa Aesar, Karlsruhe, Germany) was used for the extraction of total iodine. Single element standard stock solutions of iodine and tellurium (both 1000 mg/L) (Spectrascan, Teknolab AS, Ski, Norway) were used for the quantification of iodine. The reference material (BCR CD200 Bladderwrack) was analysed together with the samples to document the analytical quality. Similar iodine values were obtained 584±8 mg/kg (average ± standard deviation) throughout the analytical series.

For the extraction of iodine from the samples, approximately 0.2 g of homogenized sample was weighed into tubes (Sarstedt, 15 mL) and 5.00 ml Milli-Q water and 1.00 ml 25 % TMAH were added to the tubes. The tubes were then shaken using a vortex mixer and placed for 3 hours in an oven (90 °C). After 90 min the tubes were shaken using a vortex mixer and subsequently put back in the oven for the remaining 90 min of the extraction time. After cooling, the tubes were diluted up to a total volume of 25 mL with Milli-Q water (resulting in a TMAH concentration of 1 % w/w) and centrifuged (SIGMA 3-18K, Buch & Holm) at 4248 x *g* and 10 ˚C for 10 minutes.

Prior to analysis, the supernatants were diluted into the linear range of the calibration curve with 1% TMAH and the internal standard (Te) was added (10 µg/L).

The settings of the ICP-MS system (iCAP Q ICP-MS, Thermo Scientific) were 1550 W plasma power, 14 L/min plasma gas flow, 0.8 L/min auxiliary gas flow and 1.02 L/min nebuliser gas flow.

Isotopes monitored were ^{127}I and ^{125}Te. External calibration in the concentration range of 0.15–100 µg/L was used for the quantification of total iodine. Internal calibration with ^{125}Te was used to correct for potential fluctuations in the instrument.

2.4 Statistical analysis

Data are presented as average ± standard deviation. For the statistical analysis, the software PRIMER (v7) was used produced by PRIMER-e, Quest Research Limited, New Zealand. To test for differences in the means between populations one-way PERMANOVA (analysis of variance) was conducted using a 5 % significance level. To test for differences in the means between season and sites, and season and depths two-way PERMANOVA was applied. Whenever a significant difference between sample means or interaction of factors was revealed by PERMANOVA, a pairwise comparison among levels of factors was performed to compare the influence from sites, seasons, and depths on the compositions. Means were considered significantly different when levels of $p<0.05$ were obtained.

3 Results

The three cultivated kelp species were analysed for total iodine concentration. The results showed a significant variation between species, where *Laminaria digitata* had the highest iodine content with a average ± standard deviation of 5,400±1,400 mg/kg dw, *Saccharina latissima* had an average iodine content of 4,000±1,700 mg/kg dw and *Alaria esculenta* had significantly (p = 0.0001) lower levels of iodine than the two other species with 250±140 mg/kg dw (Figure 2).

Saccharina latissima had a significant seasonal variation in the total iodine concentration (p = 0.0001) with highest iodine levels during winter (January 2016; 7,500±400 mg/kg dw) and lowest during summer (June 2016; 2,600±600 mg/kg dw; Figure 3A). There was no significant variation

between the two cultivation sites (p = 0.19; data not shown in figure) or the interaction of season and site (p = 0.30) and no significant variation between the two cultivation depths (p = 0.13; data not shown in figure). Furthermore, samples collected in November 2016 from first year crop (5,100±300 mg/kg dw; n=3) and second year crop (5,100±800 g/kg dw; n=3) had similar iodine concentrations (p = 0.96; data not shown in figure).

Alaria esculenta had no seasonal variation in the total iodine concentration (p = 0.20; Figure 3B). The inter-annual variation tended to have a higher concentration in the first year of growth (144±53 mg/kg dw; 2015) compared to the second-year crop (43±15 mg/kg dw; 2016), but this was not statistically significant (p = 0.32; when June and August data was included as the only months with measurements in both years).

Laminaria digitata had no significant seasonal variation in the total iodine concentration (p = 0.10; Figure 3C) and was only sampled in the year 2016 due to its status as a weed or by-crop on the lines.

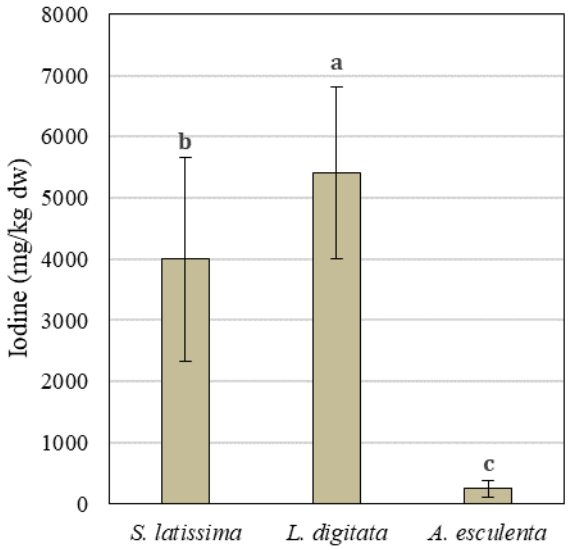

Figure 2. Iodine concentration (average ± standard deviation) for the kelp species *Saccharina latissima* (n = 65), *Alaria esculenta* (n = 30) and *Laminaria digitata* samples (n = 18) cultivated in the Faroe Islands during 2015 and 2016.

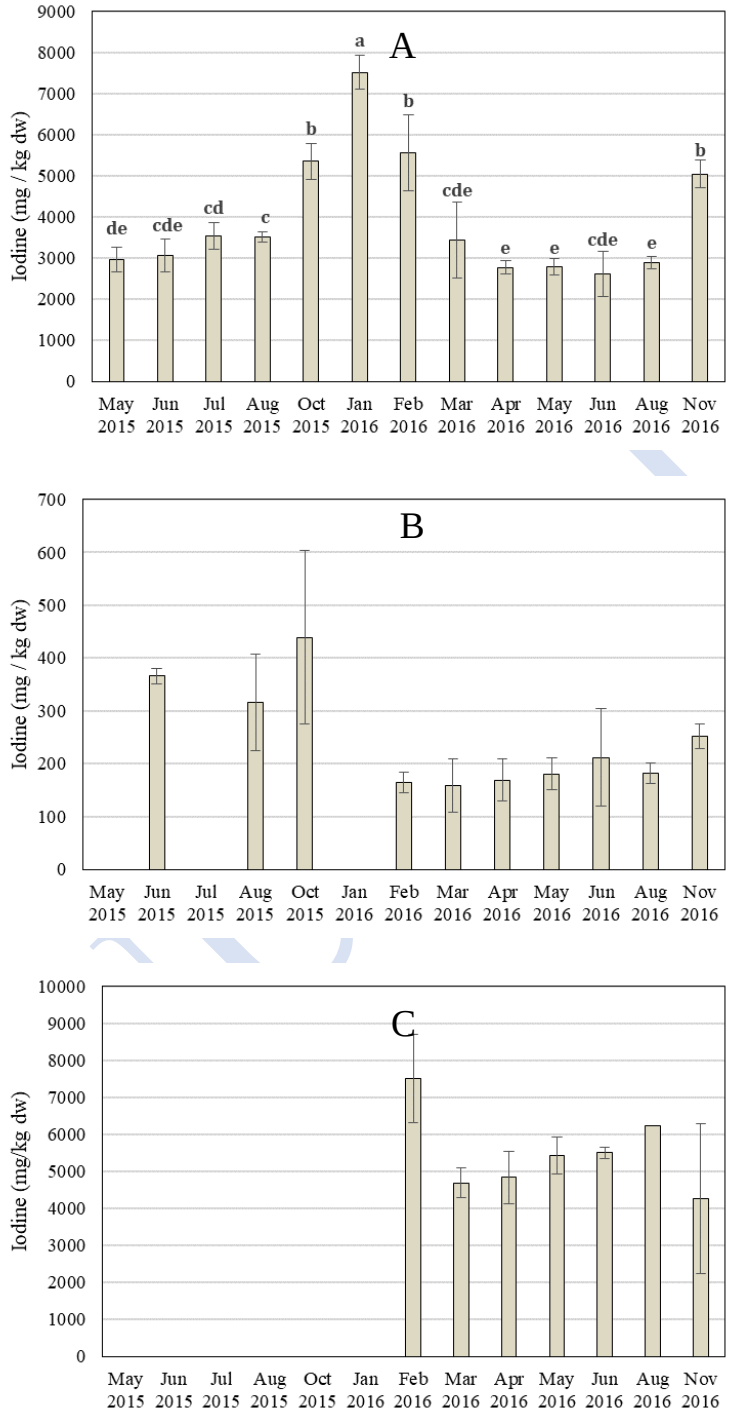

Figure 3. The seasonal variation in total iodine concentration (average ± standard deviation) for three macroalgae cultivated in the top meters (0-2 m below sea surface) on vertical lines at the wave-exposed cultivation site in Funningsfjørdur, Faroe Islands. Statistically significant seasonal variation of iodine is marked with unlike letters. *Saccharina latissima* (A) represents 39 samples in total (n=3 per month). *Alaria esculenta* (B) represents 28 samples in total (n=3 per month), except Aug. 2016 (n=2). *Laminaria digitata* (C) represents 18 samples in total (n=3 per month), except Aug. 2016 (n=1) and Jun. 2016 (n=2).

Discussion

The food items with a high content of iodine include seafood, eggs, milk, and other dairy products (Dahl and Meltzer 2009), but the highest levels of iodine are found in algae (Zimmermann 2009; Duinker et al. 2016). Brown algae of the Laminariales (kelps) are the strongest accumulators of iodine among living organisms and they represent a ca. 30.000-fold accumulation of iodine from the seawater (Ar Gall et al. 2004; Küpper et al. 2008).

Iodine might be essential to some algae, though it is mainly known to be involved in secondary metabolites processes that take up and release iodide as a mechanism to inhibit epibionts and under oxidative stress (Potin et al. 1999; Küpper et al. 2008). The distribution of iodine reaches very high levels in the more external cell layers (often exceeding 50 mM) and the peripheral tissue is consequently the main storage compartment of iodine (Küpper et al. 2008). At the subcellular level, iodine is mainly stored in the apoplasm and not in an intracellular compartment as previously proposed. This distribution provides an abundant and accessible source of labile iodine species which can be easily remobilized for potential chemical defence and antioxidative activities (Potin et al. 1999; Verhaeghe et al. 2008; Küpper et al. 2008).

The iodine content varies considerably among different macroalgal species, and generally, the highest iodine contents are found in brown (Ochrophyta) macroalgal species, whereas green (Chlorophyta) and red (Rhodophyta) macroalgal species typically have considerable lower iodine content (Duinker et al. 2016).

Our results showed that the three different brown macroalgal species had high iodine contents in the range of gram per kilo, though with a relatively large variation among species (Figure 2). The highest iodine content was found in *Laminaria digitata* with an average ± standard deviation level of

5,400±1,400 mg/kg dw, correspondingly well with concentrations previously reported in the literature (746-8,165 mg/kg dw; Jensen and Jensen 1954; Teas et al. 2004). *Saccharina latissima* also had a high average ± standard deviation iodine level of 4,000±1,700 mg/kg dw, though lower than *L. digitata*, and similar concentrations as described in the literature for this species (1,655-3,378 mg/kg dw; Duinker et al. 2016). *Alaria esculenta* had the lowest average ± standard deviation level of iodine 250±140 mg/kg dw, though still high compared to red and green species and other food sources (Dahl and Meltzer 2009; Duinker et al. 2016). The results obtained in the present study for *A. esculenta* were similar to those reported in a previous investigation (110±30 mg/kg dw; Teas et al. 2004). The lower iodine concentrations seen for *A. esculenta* could be caused by genetic and genome evolution of the family Alariaceae, as this family have not evolved as much vanadium iodoperoxidase isoforms as in the family Laminariaceae (Colin et al. 2003).

Several physical factors have been described to influence the iodine concentration, including geographical location, seasons, the part of the macroalgae used, temperature, and post-harvest storage conditions (Ar Gall et al. 2004; Teas et al. 2004). Our results showed that within the fjord the two sites had no significant variation in the total iodine concentration. Neither did the cultivation depth have a significant effect (tested from 0-10 meters below surface). The lack of variation between sites and cultivation depths might be due to 1) the stable seawater conditions characteristic for the Faroese sea (temperature and salinity), 2) a lower grassing pressure in the exposed sites (Ar Gall et al. 2004), as well as 3) a consequence of the position of growth lines. The growth lines stayed in same distance to surface at all times, and therefore never experienced large changes and high irradiance, desiccation, and atmospheric O_3 as seen for natural populations that are exposed to low tides (Ar Gall et al. 2004).

Surprisingly, no statistically seasonal variation of iodine was found for the species *L. digitata* and *A. esculenta* (figure 3B and 3C) considering the results found by Ar Gall et al. (2004) and Jensen

and Jensen (1954) showing low levels during summer and higher iodine concentrations during winter. Nevertheless, *S. latissima* had a significant seasonal variation of iodine concentrations with lower levels during summer (Mar-Aug) and highest concentrations during winter (Oct-Feb).

The age of the macroalgae (length) was earlier found to have a significant effect on the iodine concentration with the highest concentration in the young sporophytes and lower concentrations with increased age (length). In this experiment it was difficult to compare the age of *A. esculenta* as different month were monitored in 2015 and 2016, hence the results indicate that first-year algae have higher concentrations than second-year algae. For *L. digitata* the variation with age can only be seen from one year of sampling and must be considered together with the differences that are normally seen for seasons. *S. latissima* had no variation between years (i.e. age) and can be explained by the frequent harvesting (two times a year) that always leave the regrown and harvested blade tissue relatively young.

The significantly higher iodine content for *S. latissima* during winter is in the literature explained by lower oxidative stress (e.g. low grassing pressure during winter) and therefore more iodine is stored as iodide efflux and iodine volatilization is increased by oxidative stress (Ar Gall et al. 2004; Küpper et al. 2008).

This results on iodine concentration in the cultivated macroalgae enable stratified planning of the harvest for either as low iodine concentration as possible or as high as possible, depending on the end uses. Beside harvest management and selection of appropriate species, pre-treatment methods can be applied either before storage or later during food-preparation to lower the iodine content in the macroalgae. Iodine in macroalgae is highly water soluble and consequently cooking or fermentation processes can be employed to lower the final iodine concentration (Teas et al. 2004).

3.1 Recommended intake and dietary exposure of iodine

Various nutritional benefits have been associated with macroalgae. One thing that must not be forgotten, the ability of macroalgae to concentrate essential as well as toxic elements at levels that could potentially pose a risk for consumers.

There are currently no specific maximum limits or legislation for contaminants in macroalgae for food consumption (only macroalgae as a food supplement) in the European Union (EU), however in France they recommend a threshold level of 2,000 ppm (AFSSA 2009). In order to market macroalgal products, it is the responsibility of the producer to document that the macroalgae are not harmful to the health of the consumers, or unfit for human consumption. Consequently, there is a need to assess the chemical risks related to the consumption of macroalgae.

An overview of the recommended daily intake of iodine at various life stages, proposed by different organizations is presented in Table 1.

Table 1. Recommended daily intake levels and upper levels for iodine (µg/day) (WHO et al. 2007; Nordic Council of Ministers NNR 2012; EFSA Panel on Dietetic Products Nutrition and Allergies 2014).

	Population group (age, iodine intake in µg/day)					
Organization	0-5 years old	6-12 years old	12-18 years old	Adults	Lactation / Pregnancy	The upper level of safe intake
WHO	90	120	150	150	250	1000
EFSA	70-90	90	120-130	150	200	200-600
NNR	90	120-150	150	150	175-200	600

Based on a report investigating the dietary habits of the Danish population between 2011 and 2013, all age groups in Denmark had an average dietary intake of iodine above 200 µg/day. The adult males having the highest average intake (268 µg/day) corresponding to almost half the value of the upper intake level for an adult (600 µg/day) (Pedersen et al. 2015). The Danish population, therefore, has an adequate iodine intake, which might be related to the use of iodised table salt. Having said that,

an increase in consumption of macroalgae might potentially result in excessive iodine intakes above the upper levels.

Based on the obtained results for iodine in the present study, the tolerable intakes of the analysed macroalgal species have been calculated (Table 2) assuming no other iodine sources than macroalgae. These calculations show that consumption of extremely low amounts of macroalgae in the range of 0.18 g dw for *L. digitata,* 0.25 g dw for *S. latissima,* but up to 4.0 g dw of *A. esculenta* will result in an iodine intake equal to the recommended upper level of 1,000 µg iodine/day established by WHO. These findings suggest that the content of iodine could be a limiting factor for macroalgal consumption.

Table 2. Macroalgal consumption (g dw/day) corresponding to Recommended Daily Intake (RDI) and upper level (UL) iodine intake for adults according to Table 1, when macroalgae is the only iodine sources. The average annual concentrations of iodine and the highest and lowest iodine concentration was used to calculate the RDI, UL and UL with 17% bioavailability (as g dw/day) based on "average (highest – lowest) iodine concentrations" of three macroalgal species cultivated in the Faroe Islands, 2015-2016.

Macroalgal consumption *Unit: g dw/day*	**Recommended Daily Intake** average (maximum – minimum) iodine concentrations	**Tolerable upper level** average (maximum – minimum) iodine concentrations	**Upper level of safe intake with 17% uptake** average (maximum – minimum) iodine concentrations
Laminaria digitata	0.03 (0.02 – 0.035)	0.18 (0.13 – 0.23)	1.0 (0.74 – 1.3)
Saccharina latissima	0.04 (0.02 – 0.06)	0.25 (0.13 – 0.38)	1.4 (0.74 – 2.1)
Alaria esculenta	0.61 (0.34 – 0.94)	4.0 (2.3 – 6.39)	22.4 (12.7 – 35.0)

In the assessment of the food safety of macroalgae, the lack of information concerning the consumption of macroalgae products in western countries makes it difficult to propose realistic intake scenarios. The annual average consumption of macroalgae in Denmark was estimated to 34.2 g dw per capita (Susan Holdt 2017, pers. comm.). Using this estimate an average consumption of 0.1 g dw/day was calculated for a Danish citizen.

316 Average consumption rates of 0.1 g dw/day of both *L. digitata* and *S. latissima* would lead to
317 iodine intakes above the recommended daily intake of 150 µg/day for adults (WHO et al. 2007) and
318 when harvested in winter, even led to iodine intakes above the tolerable upper level of 1,000 µg/day
319 (WHO et al. 2007). A 0.1 g dw/day consumption of these two species when harvested during summer
320 would stay under the tolerable upper level of iodine intake. Therefore, it cannot be recommended to
321 consume *L. digitata* and *S. latissima* daily if small amount <0.1 g dw/day are consumed or if iodine is
322 lowered to safe levels for example by pre-treatment and/or food processing.
323 For *A. esculenta* a daily intake of 0.6 g dw per day (x6 times the Danish average daily intake)
324 would lead to an iodine exposure at recommended daily intake level and an intake of as much as 4.0 g
325 dw per day would lead to an iodine exposure at an upper tolerable level. Therefore, this species can be
326 consumed regularly without leading to any risks of adverse effects.
327 The presented intake scenarios above are worst-case scenarios in the sense that 100 %
328 bioavailability of the iodine in the macroalgae is assumed. However, a much lower bioavailability of
329 iodine from seaweed (17±2%) was recently reported from raw harvested Laminarian species at the
330 Galician coast, Northwestern Spain. The study tested iodine and bromine bioavailability by in-vitro
331 methods (simulated gastric and intestinal digestion/dialysis) (Romarís-Hortas et al. 2011).
332 When assuming 17% uptake rate, consumption of 0.1 g dw/day of *L. digitata* or *S. latissima*
333 would result in an average iodine intake of approximately 97 µg (64% of total daily intake (TDI)) or 72
334 µg (48% of TDI), respectively. Consequently, whether the species with the highest iodine
335 concentrations (*L. digitata* and *S. latissima*) are suitable for human consumption depends highly on the
336 bioavailability of iodine in these species. Under the same assumptions (17% bioavailability, 0.1 g/day),
337 *A. esculenta* would contribute with approximately 30 µg (3.4% of the TDI) to the total daily dietary
338 intake of iodine. In that case, this species could be incorporated as a stable food in a balanced diet

without any health concerns. More research is needed for a better understanding and further estimation of the bioavailability of iodine in macroalgae when consumed by humans or used in animal feed.

In addition to the bioavailability, another aspect that needs to be considered is how the macroalgae are treated prior to consumption. A recent study by Nitschke & Stengel (2016) investigated the iodine loss in macroalgae during a series of processing steps, including washing, dehydration, rehydration, and boiling. While washing and dehydration had a minor effect on iodine levels, rehydration resulted in significant losses of iodine (up to 62%) and subsequent boiling reduced the levels even further (20%). Another recent study by Stévant et al. (2018) used simple soaking treatments in warm fresh water (32 °C) to reduce the iodine in *S. latissima* to levels below the recommended 2,000 mg/kg dw by France. However, the treatments affected the nutrient content of the biomass, illustrated by considerable variations in dry matter content and the content of bioactive compounds (e.g. minerals, polyphenols, fucoxanthin). Although a considerable iodine loss during the processing steps was demonstrated, the authors concluded that daily consumption of processed macroalgae could still have implications for human health. On the other hand, the moderate consumption of these kelps will improve the iodine status in iodine-deficient populations (Nitschke and Stengel 2016; Stévant et al. 2018).

Both the iodine loss during processing and the bioavailability of the iodine in macroalgae needs to be investigated further. More knowledge of these determinants is crucial in presenting a more accurate picture of the potential health risks and nutrition benefits related to the iodine intake associated with macroalgal consumption. In fact, it is difficult to make a recommendation on human food intake of macroalgae with respect to iodine without taking into consideration the species, time of harvest, and processing of the specific food item containing the macroalgae. Especially since an

unjustified concern about the iodine content can prevent general utilisation of the healthy macroalgae in food.

4 Conclusion

The investigation of total iodine content in different macroalgal species cultivated in the Faroe Islands showed a large interspecies variation of total iodine content. The highest iodine content was found in *Laminaria digitata,* followed by *Saccharina latissima,* and a factor of 10 lower levels was found for *Alaria esculenta.* A pronounced seasonal variation of the iodine concentration was seen for *S. latissima* with lowest levels during summer and highest levels during winter. No significant seasonal variation was found for the two other species investigated.

The comparison of iodine concentrations for *S. latissima* when cultivated at two different sites, two depths and having different ages (first year vs. second-year crop) showed to have no significant impact. The biomass harvested in the lowest concentration summer months (May to August) can be considered having the same iodine concentration (approximately 3,000 mg/kg dw), though this concentration is still above the recommended threshold value.

The chemical risks related to the consumption of *S. latissima* and *L. digitata* suggest that daily intake potentially can lead to excessive iodine intakes above the established tolerable upper levels. However, the iodine intake associated with consumption of macroalgae can be decreased to a certain extent by choosing the lower iodine-containing species *A. esculenta*. Furthermore, the intake of iodine from consumption of macroalgae is highly dependent on the bioavailability of the iodine in macroalgae as well as on the post-harvest treatment of the macroalgae and/or food processing.

Increased knowledge on these topics is crucial for the advance of the European macroalgal industry and will enable authorities to communicate realistic and lucid recommendations on macroalgae consumption to the consumers.

385 The results will furthermore serve as a reference point for future development of effective pre-
386 treatment methods towards lower iodine content.
387

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Conflict of Interest

Figure 1 is also used in another publication submitted to Algal Research February 2019.

The authors declare no other conflicts of interest.

Informed Consent, Human/Animal Rights

No conflicts, informed consent, human or animal rights applicable.

Declaration of authors contribution

All persons designated as authors qualify for authorship and are listed as authors. Each author has participated sufficiently in the work to take public responsibility for appropriate parts of the content. All authors have made substantial contributions to the conception and design of the study, acquisition of data, and/or analysis and interpretation of data. All authors have drafted the article and/or revising it critically for important intellectual content. And all authors have given their final approval of the version to be submitted.

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